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Mycorrhizal availability in the basin of Lake Mills and influence on colonization and growth of Salix scouleriana under drought stress

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MYCORRHIZAL AVAILABILITY IN THE BASIN OF LAKE MILLS AND INFLUENCE ON COLONIZATION AND GROWTH OF *SALIX SCOULERIANA* UNDER DROUGHT STRESS

By Andrew Cortese

Accepted in Partial Completion Of the Requirements for the Degree Master of Science

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MASTER'S THESIS

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Andrew M. Cortese

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A Thesis
Presented to
The Faculty of
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Master of Science

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ABSTRACT

In September 2011, the removal of two dams on the Elwha River was initiated as part of the largest dam removal project in history. The drainage of Lakes Mills and Aldwell exposed 300 hectares of reservoir bottom. Reestablishment of native vegetation in the lakebeds is critical for the restoration of ecosystem function, but the reservoir sediment composition may inhibit revegetation due to poor water holding capacity. It is known that mycorrhizae can ameliorate the effects of drought stress for host plants but little is known about their availability in the Lake Mills basin. In my project, I first assessed the abundance of arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (EMF) in the Lake Mills basin. I also conducted a greenhouse bioassay in which I grew willows in potting soil and Elwha silt with different treatments of mycorrhizal inoculum. I then drought stressed the willows in order to replicate the expected summertime conditions in the Lake Mills basin. There are some viable AMF and EMF in Lake Mills, but with higher abundance near the forest and high variability in the soil. There was no effect of mycorrhizal inoculum on growth of willows and no effect of the Elwha silt on formation of AM and EM. My results suggest that willows are not dependent on mycorrhizal fungi and can establish independent of mycorrhizal propagules. Mycorrhizae can then form with willows when propagules are available, boost the mycorrhizal infectivity of the soil and then subsequently facilitate the establishment of other plant species.

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1.0 BACKGROUND

1.1 Lake Mills History

1.1.1 Dam Removal History

Two dams on the Elwha River in Olympic National Park, Washington are being removed in the largest dam removal project in American history. The dams are being removed to restore Pacific salmon runs that were decimated as a result of damming (Duda *et al.* 2008). In addition to dam removal, an ecosystem restoration project is currently in progress to reestablish native vegetation on the lakebeds and restore riparian-aquatic interactions to the lakebeds. Removal of the Elwha and Gline's Canyon dams (Lake Aldwell and Lake Mills, respectfully) will expose about 300 hectares of inundated land. Lake Mills encompasses about 200 hectares, while Lake Aldwell is 100 hectares (Chenoweth *et al.* 2011).

1.1.2 History of Lake Mills Basin

Since damming in 1925, about 13.8 million m^3 of sediment have accumulated in the Lake Mills basin (Chenoweth *et al.* 2011). The sediment is derived from shale and sandstone parent material high in the Elwha watershed (Duda *et al.* 2008). Sediment texture ranges from coarse gravel in the Lake Mills delta to extremely fine silt/clay deposits near the dam. The two main landforms that comprise the lakebed are the valley bottom and the valley wall. The valley bottom is composed of the floodplain nearest the Elwha River as well as the terraces above the floodplain (Chenoweth *et al.* 2011). The valley wall is further away from the river and features a steeper slope than the other landforms.

The terraces can be differentiated based on the proximity to the former lake shore, where the zone close to the shore is described as the boundary zone. The boundary zone was

delineated from other parts of the terraces because they featured on less sedimentation during inundation as well as higher quantities of organic material and woody debris (Chenoweth *et al.* 2011). Much of the woody debris, such as logs and stumps, is a legacy of intensive logging in the early 20th century, and some has been strategically piled into facilitation patches by the restoration team to enhance native plant establishment. The facilitation patches are intended to provide cool and moist microclimatic conditions compared to the rest of the Lake Mills basin by providing shade as well as moisture retention from the wood (Chenoweth *et al.* 2011).

1.2 Restoration of Lake Mills

1.2.1 Objectives

The main objectives for the restoration project are the reestablishment of native forest, stabilization of sediment terraces and subsequent regeneration of ecosystem function to the lakebeds (Chenoweth *et al.* 2011). The restoration team does not intend to return Lake Mills to a state identical to that of the pre-dam forest due to the massive scale of disturbance and legacy from damming (Auble *et al.* 2007; Jackson & Hobbs 2009). The restoration goals are intended to reestablish native forest to benefit threatened populations of Pacific salmon and steelhead trout in the river while concurrently inhibiting the establishment and spread of invasive plant species (Chenoweth *et al*. 2011). Revegetation is dependent upon planting of locally derived nursery stock in the lakebeds as well as natural regeneration of native plants from upland and riparian forests in the Elwha River watershed.

1.2.2 Impediments to Revegetation

Prompt reestablishment of native vegetation may be difficult because the lakebeds are not particularly conducive for plant establishment (Chenoweth *et al.* 2011; Michel *et al.*

2011). Exposed lakebeds following dam removal often feature different physical (particle texture size) and chemical (macro and micronutrient availability) characteristics than the buried soil due to the accumulation of sediment during inundation (Wells *et al*. 2008). Because the lakebed sediment composition is different than the underlying soil, the recolonizing plant community will often be different than the one that occupied the site prior to inundation (Shafroth *et al*. 2002; Auble *et al.* 2007). Sediment deposition of lakebeds during inundation is generally not homogenous. Sediment depth is usually shallower in lakeshores and deeper towards the center of the lake. Sediment texture is not homogenous and tends to be relatively fine near the dam and coarse at the upstream end near the river mouth (Wells *et al*. 2008). Such differences in sedimentation can lead to heterogenous rates of revegetation, resulting in faster recolonization of the lakeshores than other parts due to more favorable soil conditions (Hörnström 2009).

The Lake Mills basin also exhibits heterogenous patterns of sediment texture and depth. Coarse sediment, such as sand, gravel and cobble, is present near the Lake Mills delta and fine sediment, such as silt and clay, is present near the dam. The sediment depth ranges from just a few centimeters near the forest edge, which was formerly the lake shore, to tens of meters deep in the valley bottom (Chenoweth *et al.* 2011). My study will be focused on the fine deposits of silt and clay present near the Gline's Canyon dam. These sediments are very fine textured with an average diameter of 15 µm, but are also low in nutrients and have a poor water holding capacity relative to the underlying soil (Chenoweth *et al.* 2011; Cavaliere and Homann 2011; Michel *et al.* 2011). Because of the poor water holding capacity of the sediment, drought stress is likely to be the largest impediment to successful revegetation of the lakebeds. Although the Lake Mills site receives about 70 inches of rain per year, the

majority of precipitation falls in the winter and summers tend to be very dry. Average monthly precipitation from October to June is about 2.5 inches while average monthly precipitation from July to September is only 0.8 inches (WRCC, 2005). Summertime drought conditions may be common and would compound the effects of poor water holding capacity of the substrates and lead to inhospitable conditions for plant establishment (Chenoweth *et al.* 2011; Michel *et al.* 2011).

1.3 Soil Biota

1.3.1 Importance for Plant Establishment

Because the lakebeds were only recently exposed to the air, the soils do not have the composition of soil biota traditionally available for revegetation projects. Soil biota are critical for the establishment of stable plant communities both directly (symbioses) and indirectly (nutrient cycling and soil feedback loops) (Klironomos 2002; Callaham *et al*. 2008; Heneghan *et al.* 2008; Harris 2009). The organisms that symbtiotically associate with aboveground vegetation are particularly important, can be important facilitators of plant establishment (Wardle *et al.* 2004; Harris 2009), and must be taken into consideration in ecological restoration projects to ensure successful revegetation (Callaham *et al.* 2008; Heneghan *et al.* 2008).

1.3.2 Mycorrhizae

Mycorrhizae are essential for establishment of most terrestrial plants (Peterson *et al.* 2004; Smith and Read 2008). Mycorrhizae, translating to “fungus-root” in Greek, are the symbiotic relationship between plants and fungi, in which the plant fixes sugars through photosynthesis and then exchanges a portion of those sugars for nutrient uptake, drought tolerance and root pathogen resistance (Duchesne *et al.* 1988; Peterson *et al.* 2004; Sikes *et*

al. 2009). Mycorrhizae are highly diverse in both morphological and physiological characteristics; they consist of multiple phyla of the kingdom fungi and often display a broad range of functional traits between taxa (Peterson *et al*. 2004; Smith & Read 2008). The two primary types of mycorrhizae are arbuscular mycorrhizae (AM) and ectomycorrhizae (EM). Arbuscular mycorrhizae form with the majority terrestrial plant species. They are comprised of the phylum Glomeromycota and are thought to have coevolved with land plants (Simon *et al.* 1993; Taylor *et al.* 1995). Arbuscular mycorrhizal fungi form structures such as arbuscules, vesicles, and hyphae that grow within plant root cells and extend into the soil.

Ectomycorrhizae are comprised of the phyla Basidiomycota and Ascomycota and associate with the plant families Pinaceae, Salicaceae and Betulaceae (Peterson *et al*. 2004; Smith & Read 2008; Brundrett *et al.* 2009). While both AMF and EMF readily uptake labile nutrients and water from the soil (Smith & Read 2008), EMF are traditionally thought to be more effective in nitrogen uptake and can access forms that are not directly plant-available (Agerer 2001; Smith & Read 2008; Hobbie & Agerer 2009). In contrast, AMF are generally thought to be more effective in phosphorous uptake than EMF (Arias *et al.* 1990; Smith & Read 2008). However, recent work has shown that AMF are capable of accessing some recalcitrant forms of nitrogen (Whiteside *et al.* 2012). Additionally, some work suggests that EM colonized plants are able to access phosphorous as efficiently as AM colonized plants (Jones *et al.* 2008). Such research suggests that there may not be as many functional differences in nutrient acquisition between AMF and EMF as traditionally thought.

1.4 Mycorrhizae and Drought Tolerance

Arbuscular mycorrhizae facilitate drought tolerance for host plants through a combination of physical and physiological mechanisms. Arbuscular mycorrhizal colonization

can increase the root surface area of a host plant through the extension of extraradical hyphae into the soil and facilitate water uptake at lower soil matric potentials compared to non-mycorrhizal plants (Huang *et al.* 1985; Augé *et al.* 2001; Augé 2004). Additionally, extraradical hyphae by AMF can aggregate soil particles which increase the macropore area of soils and improve water holding capacity (Augé *et al.* 2001; Brady & Weil 2001; Rillig & Mummey 2006). Colonization by AMF can subsequently improve drought tolerance of host plants (Abbaspour *et al.* 2011) but the functional benefits are related to the level of colonization and amount of extraradical hyphae produced (Marulanda *et al*. 2003).

Ectomycorrhizae facilitate water uptake and drought tolerance of host plants through similar mechanisms to AM, such as by enhancing root-surface area as well as physically modifying the soil environment. Ectomycorrhizal infection results in more pronounced changes to root architecture through the formation of a mantle and Hartig net (Smith and Read 2008). Colonization by EMF can result in increased root cell volume, stem water potential and root hydraulic conductance for host plants (Landhäusser *et al.* 2002; Muhsin and Zwiazek 2002; Luo *et al.* 2009). Host plant photosynthetic rates can increase up to ten-fold as a result of improvements in water uptake (Parke *et al*. 1983). There is significant functional variability within EMF and some are more resistant to drought than others (Agerer 2001; di Pietro *et al.* 2007) which may lead to different host plant responses to drought, depending on the EMF colonizing the plant.

1.5 Dual AM/EM Host Plants

The majority of mycotrophic plants form either AM or EM associations singularly, but certain plants concurrently associate with AM/EM, making them dual-host plants. The

genera *Alnus* (Betulaceae), *Salix* and *Populus* (Salicaceae) have been shown to simultaneously form AM and EM associations under natural conditions (Gehring *et al.* 2006; Lodge 1989; Smith *et al.* 1998). However a variety of factors including the environment, plant age and access to inoculum can influence the proportion of AMF and EMF fungi that colonize a host plant (Ashkannejhad & Horton 2006, Gehring *et al.* 2006, Lodge & Wentworth 1990). In plants colonized by both AMF and EMF, AMF tend to colonize long lateral roots while EMF tend to colonize short roots. It is believed that short roots can also be colonized by AMF and then subsequently displaced with the colonization of EMF (Wagg *et al.* 2008). Arbuscular and ectomycorrhizal fungi can also offer differential benefits to the host plants. In dual-host plants AMF colonization can be important in providing short term effects on root growth, nutrient acquisition and water uptake for young plants while EMF can be more important for long term benefits (van der Heijden 2001).

1.5.1 AM and EM interactions in dual-host plants

Dual AM and EM colonization can have a positive influence on host plant growth rate compared to single AM or EM colonization (Misbahuzzaman & Newton 2006; Chen *et al.* 2000). There is evidence that competitive interactions between AMF and EMF can occur within a single host plant root. *Populus* grown under water stressed conditions (drought and flood) were found to have higher colonization of AMF than *Populus* grown under mesic soil conditions, which had higher colonization of EMF (Gehring *et al.* 2006). Such results suggest that EMF can displace AMF for favorable growing conditions as a function of competitive exclusion (Lodge and Wentworth 1990).

1.6 Mycorrhizae in Ecological Restoration

1.6.1 Applications

The addition of mycorrhizal inoculum to restoration projects can be beneficial for the re-establishment of vegetation, especially in cases where the disturbance destroyed mycorrhizal networks in the soil, and natural sources are not close enough to the site to facilitate natural inoculation. Some research has examined the addition of mycorrhizal inoculum in ecological restoration projects and found positive effects on plant growth compared to uninoculated plants, particularly in sites with low nutrient availability in the soil (Johnson 1998; Richter & Stutz 2002; Allen *et al.* 2003). Because of the low availability of nitrogen and phosphorous in Lake Mills substrates, mycorrhizal inoculation may be critical to the reestablishment of native vegetation.

1.6.2 Mycorrhizal Inoculum Sources

Mycorrhizal fungi can be reintroduced to a site via whole-soil, commercial, or greenhouse propagated inoculum (Allen *et al.* 2003; Enkhtuya *et al.* 2003; Corkidi *et al.* 2004). Whole-soil inoculum uses soil from an undisturbed site and contains both mycorrhizal fungi as well as the suite of other soil organisms and, potentially, soil borne pathogens. Whole-soil inoculum is widely accepted to be the most effective in reestablishment of mycorrhizae in a site (Corkidi *et al.* 2004; Corkidi *et al.* 2005). One reason is that it includes the entire soil community in addition to mycorrhizal fungi including bacteria, non-mycorrhizal fungi and microinvertebrates rather than just a few species of fungi. These organisms can interact and produce soil feedback loops that may stimulate the formation of mycorrhizae (Klironomos 2002; Wardle 2004; Callaham *et al.* 2008; Heneghan *et al.* 2008). Often these organisms, in addition to the mycorrhizal fungi, are absent from disturbed areas and may be critical to the restoration of soil in its entirety (Halpern *et al.* 2007; Harris *et al.*

2009; Wardle *et al.* 2004). There are few reports of commercial inoculum being more effective than whole soil inoculum (Enkhtuya *et al.* 2003), but most studies have reported the opposite (Hung & Molina 1986; Corkidi *et al.* 2004; Corkidi *et al.* 2005). Often the effectiveness of commercial inoculum is variable and can be dependent on the manufacturer, batch and ambient storage conditions (Hung & Molina 1986; Corkidi *et al.* 2005). Commercial inoculum was used to inoculate plants grown in silt from Lake Mills prior to dam removal but did not form mycorrhizae (Cook *et al.* 2011).

1.7 Mycorrhizal Fungi in Lake Mills

1.7.1 Availability

There are two studies that have examined the abundance or infectivity of mycorrhizal inoculum present in Lake Mills. A sample of 4 replicate AM Spore extractions prior to dam removal found an average of 12±9 (standard error) spores per gram of soil (Chenoweth *et al.* 2011). Although the spores appeared turgid and healthy, it was unconfirmed whether the AM spores in Lake Mills were actually viable. Cook *et al.* 2011 grew plants in silt from Lake Mills but did not detect any mycorrhizal colonization. There were no data regarding the distribution of AM spores in Lake Mills or the presence or absence of ectomycorrhizal spores. It is likely that the lakebed, now exposed to the atmosphere, will have an influx mycorrhizal fungal spores dispersing from the forests adjacent to Lake Mills. There are many documented cases of arbuscular and ecto-mycorrhizal spores being vectored long distances by wind and animals (Ashkannejhad & Horton 2006; Trappe & Maser 1976; Warner *et al.* 1987). However, such processes may not provide adequate mycorrhizal inoculum within the timespan required by the revegetation project on Lake Mills. Thus, artificial inoculation of restoration plantings may be required to reestablish vegetation in some or all of Lake Mills.

1.8 Research Objectives

1.8.1 Objective 1: Assessment of Mycorrhizal Availability in Lake Mills Basin

- Assess the distribution of AM spores
- Confirm viability of AM spores in sediment
- Assess availability of EMF

1.8.2 Objective 2: Effect of Elwha Silt, Drought and Mycorrhizal Inoculation on Willow Growth and Formation of Mycorrhizae

- Determine if mycorrhizal inoculum is effective in Elwha silt
- Compare mycorrhizal colonization rates between Elwha silt and other inoculum types
- Look for effect of mycorrhizal inoculation on growth of willows in Elwha silt

2.0 RESEARCH DESIGN

2.1 Mycorrhizal Availability in Lake Mills

2.1.1 Quantification of Arbuscular Mycorrhizal Spores

My research project first quantified the abundance of AM spores in the Lake Mills basin. Any spores in the sediment will be the primary source of inoculum for restoration plantings from which mycorrhizae will form. My objective was to quantify the distribution of spores in Lake Mills and establish a baseline of AMF abundance in Lake Mills.

2.1.2 Survey of Ectomycorrhizae

Because EMF spores are much smaller than AMF spores, there is no way to directly extract them from the soil. As way to qualitatively assess the presence of EMF in Lake Mills, I sampled a small number of naturally regenerated willow seedlings and examined their roots

for signs of EMF colonization. I sampled one of the designated control plots that were neither planted nor treated with fertilizer to prevent collection of planted stock. I used molecular methods to confirm any samples as being EMF and identify the fungus.

2.2 Drought Tolerance Bioassay

2.2.1 Host Plant Selection

My research project also tested the effect of mycorrhizal inoculum on the drought tolerance of Scouler's willow (*Salix scouleriana*) grown in reservoir sediment in a greenhouse bioassay. I used *S. scouleriana* as my bioassay plant species because it is abundant in the Elwha watershed, grows rapidly and is a known dual-host that forms EM and AM associations readily (Lodge 1989; Smith *et al*. 1998). Additionally, *S. scouleriana*, like other willows, is capable of aggressive vegetative reproduction as well as prolific seed dispersal; the seeds are highly mobile and can be widely dispersed via wind and water (Brown & Chenoweth 2008). As of March 2012 there was a high abundance of *S. scouleriana* and *S. sitchensis* natural regeneration establishing in the former shore of Lake Mills, indicating that they will likely play a large role in the revegetation of Lake Mills.

2.2.2 Mycorrhizal Inoculum Sources

My bioassay used multiple treatments of natural and commercial inoculum types which represented different sources that could be used by the revegetation team on Lake Mills. Natural inoculum treatments included soil from a riparian willow stand, mature upland forest as well as nonnsterilized reservoir silt from the boundary zone in Lake Mills. The Elwha silt treatment represents the mycorrhizae that are currently present in Lake Mills. Commercial treatments included a pure culture of an AM fungus *Glomus intraradices* (Mycorrhizal Applications' MycoApply®) and a mixture of multiple AMF, EMF as well as

other soil microbes (Fungi Perfecti's Myco-Grow®). My research addressed which mycorrhizal inoculums are infective in reservoir silt from Lake Mills, and whether they provide enhanced drought tolerance for willows grown in the silt, compared to non-mycorrhizal control and Elwha silt-inoculated plants. My drought stress bioassay attempted to replicate summertime drought conditions in the lakebeds and address whether mycorrhizae can form in the silt, if there are viable AMF and EMF in sediment from Lake Mills, and if mycorrhizal inoculation influences growth and drought tolerance of willows. Additionally, I grew some willows in potting soil in an attempt to compare and contrast colonization levels of willows grown in both soils. The objectives of my greenhouse bioassay are to assess whether any mycorrhizal fungi present in Lake Mills are viable and, if so, if the formation of mycorrhizas is inhibited by Elwha silt. Also, I want to determine whether there is a functional effect of mycorrhizal inoculation on the drought tolerance, as measured in root and shoot biomass as well as foliar nitrogen and phosphorous, of willows grown in Elwha silt.

4.0 METHODS

4.1 Study Site

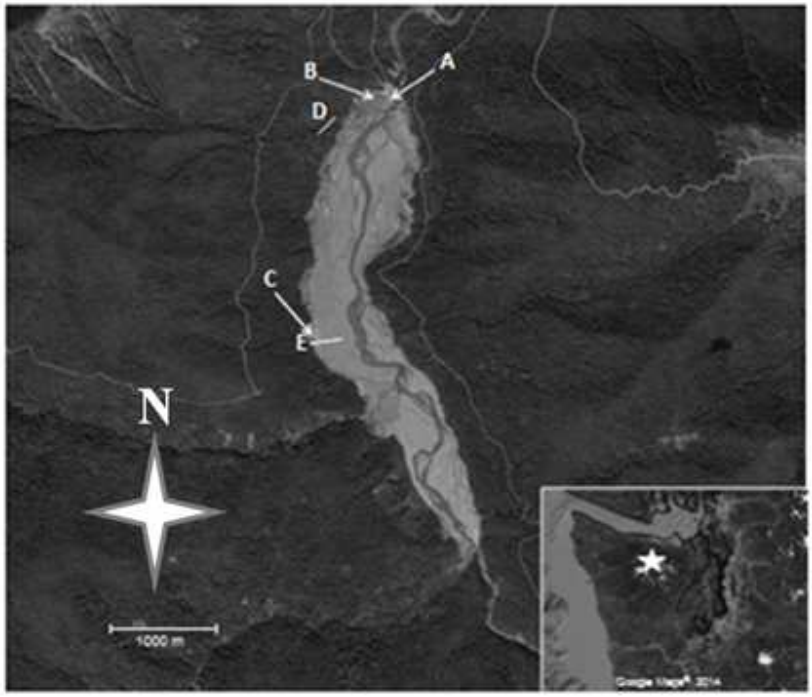

Figure 1: Map of Lake Mills basin with location in Washington State shown for reference. Point A shows the location of the Gline's Canyon dam. Point B shows the location where I collected Elwha silt inoculum. Point C shows the location where I collected willow seedlings for assessment of EM availability. Point D shows the location of the transect for collection of mature forest soil inoculum. Point E shows the location of the transect for collection of Elwha soil for AM spore extractions.

4.2 Quantification of AM Spores in Lake Mills

4.2.1 Field Methods

Fine sediment samples were taken from the northwest section (Figure 1E: 47.983646 N, -123.603469 W) of Lake Mills along a 376 meter transect from the lakeshore to the bank of the Elwha River on March 6, 2012. Three distinct zones, as delineated by the restoration team at ONP, were sampled: the boundary zone closest to the forest edge (7-87 meters), followed by the planted zone-which had been planted by the restoration team (107-227

meters), and the unplanted zone which was furthest from the forest (291-376 meters). Five samples each were collected from the boundary and planted zones and four samples were collected from the unplanted zone. Samples approximately 10 cm wide by 20 cm deep were collected with a trowel and placed into a labeled Zip-Loc® bag and stored at 4°C until extraction took place.

4.2.2 Spore Extraction

Spore extractions were conducted in September and October 2012, primarily following the sucrose-extraction methods of Allen *et al.* 1979. Five replicate spore extractions were conducted for each sediment sample to account for a heterogeneous distribution of spores and improve the accuracy of the counts. Five g of wet soil were a placed in a pitcher and then agitated with pressurized tap water until filled to 1 liter mark. Sample was allowed to settle for 10 seconds and was then poured into nested 1 mm and 25 µm sieves to remove large sediment and organic material while trapping spores in the 25 µm sieve. The contents of the 25 µm sieve were washed into a 50 ml scintillation vial with distilled water. Tube was then filled to 35 ml and then centrifuged for 4 minutes at 2500 RPM in a Thermo Scientific CL2 Centrifuge. After centrifugation, the spores and sediment particles formed a pellet in the bottom of the vial, from which the excess liquid was decanted until 10 ml of supernatant remained. Tubes were then filled to 50 ml with a 60% sucrose solution and then centrifuged again for 4 minutes at 2500 RPM. Sucrose solution was then decanted into a 25µm sieve and rinsed with distilled water to prevent desiccation of the spores. Spores were then washed into a clean Petri dish and then rinsed into a Fisherbrand 25 mm Glass Microanalysis Vacuum Filtration Setup. Sample was filtered with distilled water onto 25 mm GE Magna 5 µm membrane nylon filter and then stored at 4°C.

4.2.3 Spore Quantification

Using an OlympusSZ51dissecting microscope, at 40 x magnification all AMF spores were counted. Spore counts ranged from 1-130 spores per filter paper. Arbuscular mycorrhizal spores were identified by their spherical, turgid appearance; any elliptical or flaccid spores were assumed to be non-AM or dead, respectively (INVAM, 2014). To standardize spore densities to number per gram of dry soil, 10 g of each sample were dried for 48 hours at 90°C and the number of spores were converted from wet soil counts to the density of spores per g of dry soil.

4.3 Survey of Ectomycorrhizal Fungi in Lake Mills

4.3.1 Field and Laboratory Methods

I collected four naturally regenerated willow seedlings from a control plot in Lake Mills on 6/8/2013 (Figure 1C: 47.996743 N, -123.606473 W). Plants were selected at random and carefully removed with the root system intact. Plants were taken back to WWU and stored overnight at 4°C. Under 40x magnification using an OlympusSZ51 dissecting microscope, I picked EM colonized root tips off from the root system and then stored them in Eppendorf tubes at -18°C until genetic analysis.

4.3.2 Genetic Analysis

Ectomycorrhizal root tips were placed in 2 x CTAB solution and sent to Tom Horton's lab at SUNY ESF for genetic sequencing. DNA extraction, polymerase chain reaction (PCR), and amplification of restriction fragment length polymorphisms (RFLP) types were conducted using a modified glassmilk protocol sensu Nuñez *et al*. 2013. The primers ITS1f (White *et al.* 1990) and NLB4 (Martin & Rygiewicz 2005) were used to amplify the fungal nuclear ribosomal internal transcribed spacer (ITS) region. Amplicons were digested using the restriction enzymes *Hinf*I and *Hae*III (New England Biolabs,

Ipswich, MA) following the manufacturer's protocol, and then visualized restriction fragment patterns on 3% agarose gels sensu Gardes & Bruns 1996. For each morphotype, the ITS region was re-amplified from DNA extracts then sequenced them on an ABI 3730xl in one direction using ITSf1as the sequencing primer. Sequences were grouped into operational taxonomic units (OTUs) in Mothur 1.31 (Schloss et al. 2009) using a cutoff of 97% sequence similarity, not counting end gaps and treating internal gaps as a single character. We named OTUs based on BLAST comparisons to GenBank: we considered a sequence conspecific with named GenBank sequences at >97% similarity across the available ITS region.

4.4 Drought Stress Bioassay

4.4.1 Soil Collection and Sterilization

Elwha silt was collected from the Lake Mills basin, Olympic National Park on October 5, 2012. Silt was collected from the boundary zone 10 m east of the boat launch in Lake Mills, approximately 200 m southwest from the Glines Canyon Dam (Figure 1B: 48.002170 N, -123.601602 W). I chose to collect silt from this part of Lake Mills because it consists of the fine textured sediment that covers the majority of the lakebed and represents the most stressful growing environment for plants (Chenoweth *et al.* 2011). Approximately 31 gallons of silt were collected from the upper 20 cm of the deposit with a shovel and stored in 18 gallon totes. Sediment was brought to WWU and mixed in a 1:1 silt:sand ratio with coarse sand (3-4 mm average diameter) from Salazar's Greenhouse Supply in Burlington, WA. The mixture was treated in the Biology Department Pro-Grow SST-60 soil sterilizer for 4 hours at 200°F, and then repeated after a 24 hour resting period. A second soil medium of potting soil was used to evaluate the effects of the Elwha soil on formation and function of mycorrhizae. The potting soil contained a 6:1:1:1:1 ratio of sand, topsoil, mushroom

compost, horse manure and sawdust, respectively. The mixture was sterilized following the same protocol as the Elwha silt. Both soils were stored in sealed totes at 20°C until planting on November 14, 2012.

4.4.2 Mycorrhizal Inoculum

Three sources of native and two sources of commercial inoculum were used in my drought tolerance experiment. Mature forest soil inoculum was collected from a mature mixed Douglas-fir (*Pseudotsuga menziesii*) and willow soil inoculum was collected from a Scouler's willow stand. A pure AM culture and mixed AM/EM culture were used as commercial inoculum treatments.

Mature forest whole-soil inoculum was collected from Olympic National Park on October 5, 2012. Inoculum was collected from the mature Douglas-fir/hardwood forest on the west side of Lake Mills near the boat launch site (Figure 1D). Samples were collected with a trowel every 10 m along a 100 meter north-south transect. Approximately 500 ml of soil were taken from a 10 cm wide by 20 cm deep hole in the interface of O and A horizons from each point, for a total of 5 liters of soil. Inoculum was stored in gallon Zip-Loc® bags at 4° C until November 14, 2012. Willow whole-soil inoculum was collected on November 12, 2012 from a native population of Scouler's willow at the confluence of the North and South forks of the Nooksack River (48.807203 N, -122.201715 W). Inoculum was collected with a trowel from 10 randomly selected points, ranging from 5-20 cm deep from two patches of willows until 5 liters were collected. Inoculum was stored at 4°C until use on November 14, 2012. Elwha silt inoculum was taken from the silt collected for growing medium on October 5, 2012. The arbuscular mycorrhizal (AM) treatment consisted of a pure culture of *Glomus intraradices*, sent by Mycorrhizal Applications® in Grants Pass, OR.

Myco-Gro© (Fungi Perfecti ®, Olympia, WA) was used as the dual AM/EM (FP) treatment, which contained a combination of various AM and EM fungi.

4.4.3 Inoculum Application

I used 50 ml of mature forest soil, willow soil and unsterilized Elwha silt (30g, 40g and 30g, respectively) for each replicate. I used 50 ml because that amount was sufficient to form a 1 cm deep layer on the rooting zone and ensure that the roots would come into contact with the inoculum. For commercial inoculum, I attempted to match the concentration recommended by the manufacturer to ensure that infectivity levels were not biased towards one treatment. I used 3g of AM inoculum per AM replicate and 1g of AM/EM per Fungi Perfecti® replicate. To account for any physical and chemical characteristics of inoculum on willow growth, I created a mixture of all inoculum types and sterilized it in the autoclave at 121° C at 15 psi for 90 minutes, followed by a 24 hour rest and a repeat autoclave to kill off any persistent spores. The mixture was comprised of 50g of AM, 42g of AM/EM, 1200 g of willow and 1200g of mature forest soil. Each replicate received 40 g of sterilized inoculum mixture.

4.4.4 Plant Propagation

Willow stakes were collected October 16, 2012 from the riparian zone at the confluence of the North and South forks of the Nooksack River near Van Zandt, WA (48.807203, -122.201715). The willows were growing in sandy soil with a mixture of cottonwood (*Populus trichocarpa*), alder (*Alnus rubra*) as well as exotic knotweed (*Polygonum cuspidatum* x *bohemicum*) and blackberry (*Rubus armaenicus*). Willow shoots were chosen based on size and appearance of high vigor; willow shoots 0.5-2 cm in diameter with green foliage and clear stems, lacking cankers or other signs of disease were selected.

Willows were wrapped in moist newspaper and stored in garbage bags overnight to inhibit desiccation. Willows were processed on October 17, 2012; stakes were cut to 10 cm with a pull-saw, with a minimum of 2 bud nodes, and then surface sterilized for 20 minutes in 3% H_2O_2 followed by a triple rinse in tap water. Stakes were then weighed and planted in a 73.6 x 45.7 x 15.2 cm tote in sterilized vermiculite on October 17, 2012 and watered every other day for 4 weeks to promote rooting.

After four weeks, enough stakes had sprouted to allow an adequate number of replicates for each treatment. Plants were randomly sampled from the tub and were then transplanted into 1 liter (12 cm diameter x 11 cm height) greenhouse pots (Dillen Products Middlefield, OH). Pots were filled with 3cm of sterilized soil, followed by a layer of sterilized and live inoculum. Rooted cuttings were placed in the pots, ensuring contact of roots with live inoculum, and were then subsequently filled with sterilized soil. After planting, the number of shoots and the height of the longest shoot were recorded for each replicate. Twelve unused plants were harvested and dried for initial nutrient analysis measurements to compare with the nutrient content of the final harvest plants.

4.4.5 Microbial Wash

I included a microbial wash control to isolate the effect of mycorrhizae from any effects of soil bacteria present in whole-soil inoculum (Koide & Li 1989) The wash was prepared from 1200g of willow and 1200g of mature forest soil on 11/13/2012. Samples were first individually passed through a 4 mm sieve to remove large debris, and were then placed into separate 1 liter beakers. Beakers were filled to 1 liter with distilled H_2O and stirred for 1 minute with a spatula to suspend fine particulates. After agitation, samples were sequentially passed through 1 mm, 250 μm, 125 μm, and 25 μm sieves to remove fine particulates and

AM spores. A Gast Laboratory 23 Series Rotary Vane Vacuum Pump (model 0523 V4F G588DX). I used a 1 L Erlenmeyer flask with a vacuum attachment to a 150 mm Buchner funnel. Whatman® 150 mm size 1 and 3 filter paper was used for filtration down to 11 μm and 6 μm pore sizes, respectfully, to remove EM spores, which range from 6 μm to 15 μm in size. Samples were filtered 3 times through the 11 μm paper and 5 times through the 6 μm paper to ensure no EM spores passed through. The two samples were then combined and refrigerated overnight in sterilized 1 liter Nalgene® bottles. After planting, 20 ml of microbial wash were added to each pot.

4.4.6 Watering Regime/Drought Stress

Plants were watered to container capacity every third day as well as misted with an overhead watering system three times a week from 11/14/2012 to 1/25/2013. Plants were given adequate water in order to allow mycorrhizae to establish, as well as to allow plants to recover from transplanting shock. Drought stress was conducted for 8 weeks between 1/15/2013 and 3/15/2013 by turning off the overhead watering system, watering plants to container capacity and subsequently increasing the watering interval by 1 day until plants reached wilting point. The watering interval was designed so the plants' apical tissue lost turgidity before the next application of water. Because the two growth mediums had different water holding capacity, potting soil replicates were watered approximately every fourth day while the Elwha replicates were watered weekly. Plants were randomized every 30 days to minimize confounding variability due to greenhouse microclimate. Average ambient temperature was 19°C and average relative humidity was 56%. However, every other day, humidity fluctuated between 30-70%, most likely due to the overhead watering system turning on for other greenhouse benches. Artificial lighting was used to supplement natural

sunlight; average light intensity was 770 lux for 16 hours per day. Light and moisture data were collected with a LoggerLite 1.6.1 data logger.

4.4.7 Plant Harvest

Willows were harvested on 3/25/2013 after 16 weeks of growth. Plants were gently removed from the pots, and soil was washed away from the root system. Removing plants from the dense Elwha soil was difficult and some roots were broken during harvest. To maximize root recovery, root fragments were picked from the soil for 5 minutes per replicate. Roots were subsampled for AM analysis. Total and subsample wet weights of roots were recorded and stored in distilled water at 4°C until EM analysis. Following EM analysis, roots were dried at 60°C for 48 hours and then weighed again. Wet and dry weight data were used to create regression that allowed the dry weight of subsamples to be estimated, and thus total dry weight to be estimated. The number of stems and leaves were recorded immediately post-harvest. Shoots were placed in envelopes and dried in a Thermo Scientific HeraTherm® drying oven at 60°C for 48 hours.

4.4.8 Ectomycorrhizal Assessment

Ectomycorrhizal colonization was assessed via direct examination of 10 randomly selected wet root segments from each plant. The entire root system was cut into 5 cm sections and placed into a 200 ml beaker of distilled water. Using forceps, roots were stirred until all segments were suspended and then 10 were randomly selected. Under 40 x magnification using an OlympusSZ51 dissecting microscope, each root tip was picked off and then determined to be either EM or non-mycorrhizal. Root tips were counted, and all EM were sorted and counted by morphotype. Percent colonization was calculated by counting the number of colonized root tips and dividing by the total number of root tips. Colonized root

tips were placed in 15 ml Eppendorf tubes and then frozen at -18°C. Root tips were then preserved in CTAB and sent to Tom Horton's lab at SUNY ESF in Syracuse, NY for genetic analysis.

4.4.9 Arbuscular Mycorrhizal Assessment

Arbuscular mycorrhizal colonization was assessed through clearing and staining roots (sensu Koske and Gemma 1989). Five randomly selected sections of each plant's roots were sampled and cleared and stained. Roots were cleared in 2.5% KOH at 20°C for 7 days. Roots were then triple-rinsed in distilled H_2O and then acidified with 3% HCl for 8 hours. Roots were then stained for 24 hours with 0.5% Trypan Blue. After staining, 12 root sections approximately 2 cm long were placed on a microscope slide and covered with lactoglycerol and a cover slip. Under 200x magnification using a Nikon Eclipse 80i compound microscope, a total of 144 root intersections (root segment meets crosshairs in ocular micrometer) were examined for mycorrhizae as well as root pathogens for each replicate sensu McGonigle *et al*. 1990. Hyphae that were greater than 5 µm, lacked septa and were lumpy in appearance (Rillig *et al.* 1998) were identified as AM hyphae. Other AM structures counted were arbuscules and vesicles. Together, I used these structures to calculate percent AM colonization. Basidiomycete and ascomycete hyphae were counted as a complementary metric to the root tip counts for percent colonization of EM. Basidiomycete and ascomycete (BA) hyphae were septate and less contorted than AM hyphae. The presence of clamp connections in a hypha was diagnostic of a basidiomycete, but the lack of clamp connections meant that a hypha could have been from an ascomycete or basidiomycete fungus (Peterson *et al*. 2004). Dark septate endophytes (DSE) were also observed and counted. Septate hyphae exhibiting a contorted appearance were diagnostic of DSE's. Additionally, DSE's often

appeared brown under the microscope because they generally would not pick up the Trypan blue stain as readily as basidiomycete and ascomycete hyphae (Jumpponen & Trappe 1998).

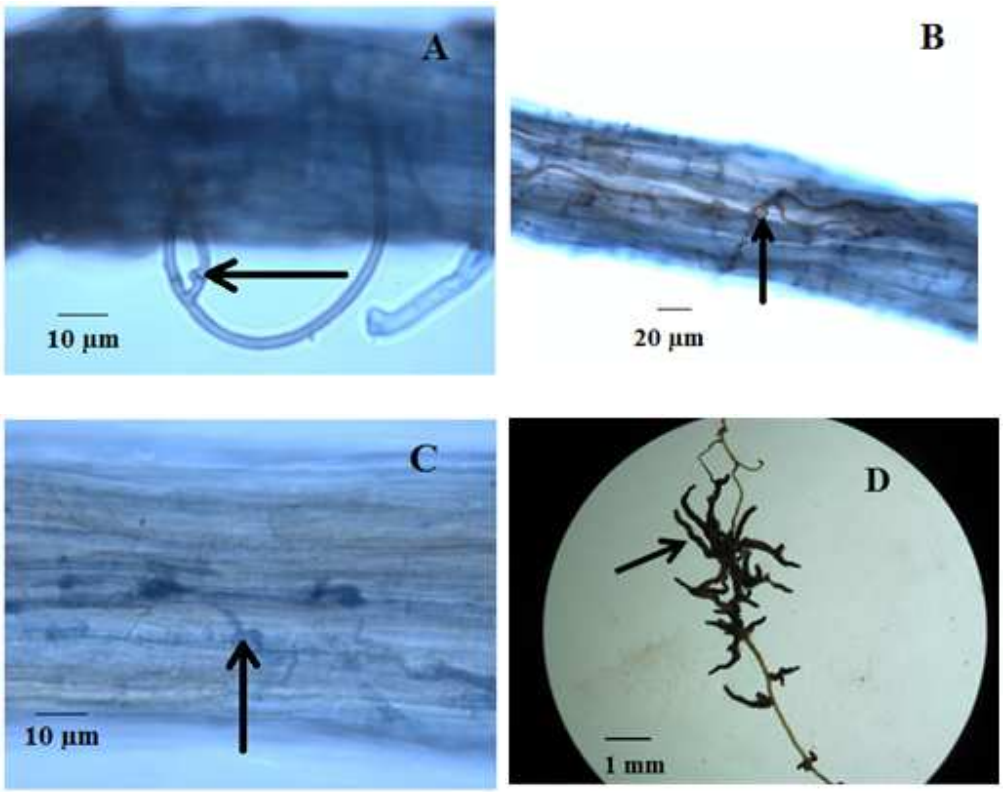

Figure 2 A-D. Images from cleared and stained roots under compound microscope (200-400x). Image A= clamp connection (arrow) indicative of basidiomycete hypha. Image B= dark septate endophyte (arrow). Image C= AM hypha (arrow). Image D= ectomycorrhizal colonized root tip (arrow).

4.4.10 Nutrient Analysis

Dried willow foliage samples were ground to <0.5 mm particle size in a Foss Cyclotech 1093 Sample Mill and sent to Kansas State University's Research and Extension soil testing laboratory in Manhatten, KS in October 2013.

4.5 Statistical Analysis

Statistical analyses for spore counts, mycorrhizal colonization and nutrient analysis were conducted in R (Stats package: version 2.15.1; R Core Team, 2012). Spore counts were $\log_{10}$ transformed and analyzed using spore density by distance from forest edge in linear regression. Mycorrhizal colonization was compared with one-way analysis of variance (ANOVA) (α=0.05). All data fulfilled assumptions of homogeneity of variance and independence. Bartlett's test was used to test homogeneity of variance and Shapiro-Wilk's test was used to test normality of distribution (Stats package: version 2.15.1; R Core Team, 2012). Mycorrhizal colonization data for the AM treatment slightly violated the assumption of normality with heavy tails but ANOVA was carried out because a slight violation of normality would not significantly affect the results (Zar 2010). Pairwise comparisons were carried out with Holm's adjusted pairwise t-tests (α=0.05) (Stats package: version 2.15.1; R Core Team, 2012). Percent and total nitrogen and phosphorous between treatments were compared using one-way ANOVA (α=0.05). All data fulfilled assumptions of normality and homogeneity of variance. Willow biomass between treatments was tested with a one-way analysis of covariance (ANCOVA) using SPSSX (IBM, 2013). Bonferroni adjusted t-tests were used for pairwise comparisons between treatments (α=0.05).

5.0 RESULTS

See appendix for supplementary figures and tables.

5.1 Mycorrhizal Availability of Lake Mills

5.1.1 AM Spore Counts

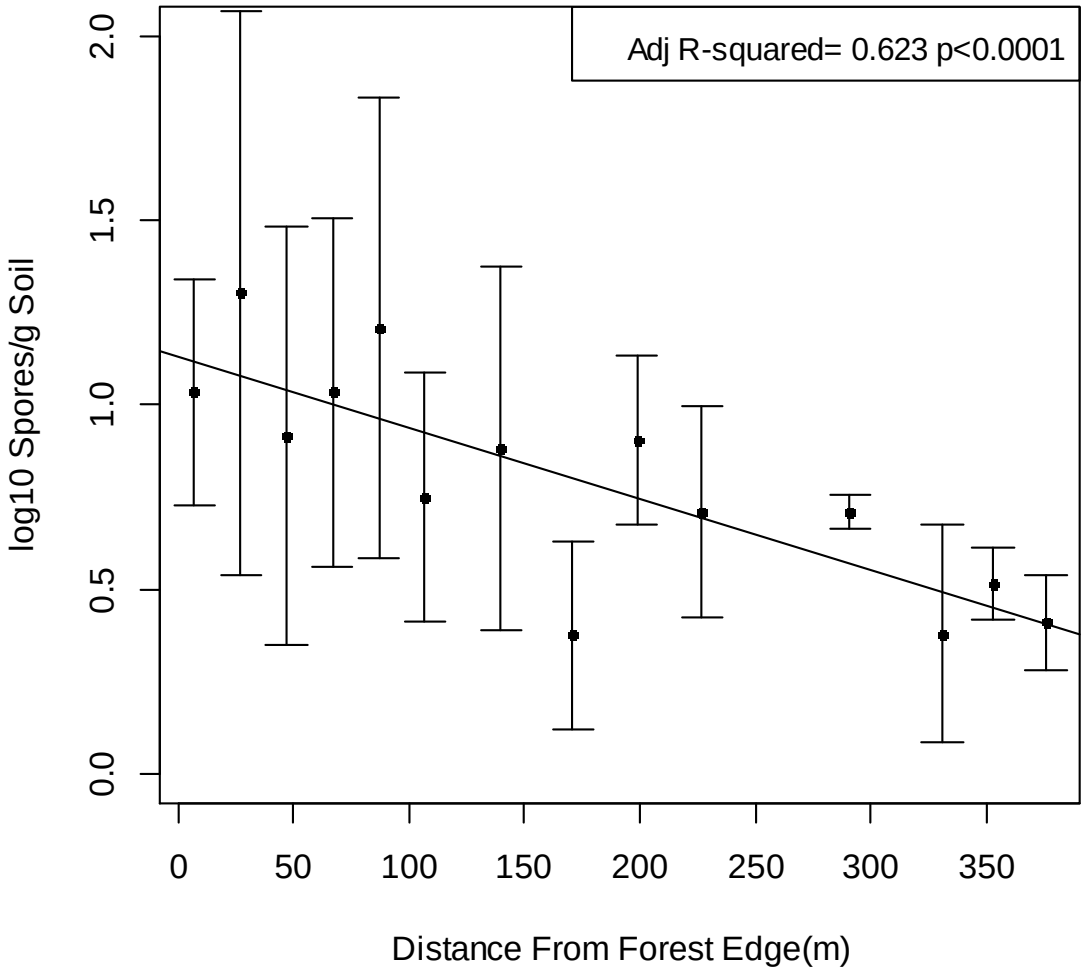

Figure 3: Log_{10} density (spores/dry g soil) of arbuscular mycorrhizal spores present in the basin of Lake Mills along one transect from forest edge to main channel of the Elwha River, March 6 2012 (Figure 2E). Each point represents the average of 5 replicate spore extractions and error bars represent standard error.

Arbuscular mycorrhizal spore density showed a negative relationship with distance from the forest edge (Figure 3). Each point on the figure represented the average density of five replicate spore extractions from one sediment sample, in which there was high variability between replicates. Average standard error within samples was ±11.21. Data were $\log_{10}$ transformed to account for decreasing variance of spore counts with distance from the forest edge.

5.1.2 Mycorrhizal Inoculum Potential of Elwha Silt

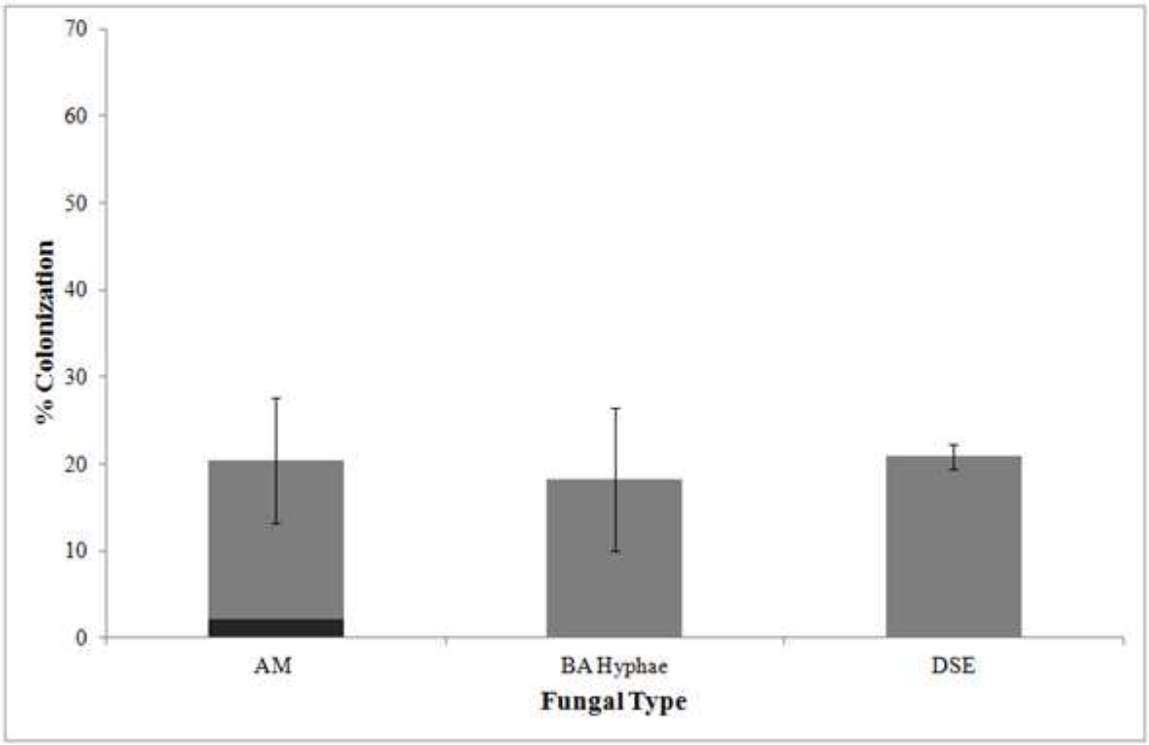

Figure 4: Fungal colonization of willows inoculated with reservoir silt from Lake Mills and grown in greenhouse bioassay. Error bars indicate standard error. AM=arbuscular mycorrhizal colonization; hyphae is light grey while arbuscules and vesicles are dark grey. BA=basidiomycete/ascomycete hyphae. DSE= Dark septate endophytes. No ectomycorrhizal root tips were observed.

Soil from the Lake Mills basin used to inoculated willows in my bioassay contained viable AMF, basidiomycetes and/or ascomycetes as well as DSE's. Colonization was approximately 20% for all fungi but with high variability for AM and BA hyphae (±10% SE) (Figure 4). No fully colonized EM root tips were observed, but under 40x magnification there appeared to be incipient mantle formation. BA hyphae were present but could not be confirmed as EM because the fungal trophic status could not be determined, and the fungus may have been non-mycorrhizal. No colonized EM root tips were observed on Elwha silt inoculated plants. Despite not being statistically different ($p>0.05$), Elwha silt inoculated plants had about 9 times as much BA hyphae as the non-mycorrhizal control (Figure 5).

5.1.3 Ectomycorrhizae from Lake Mills

Field collection of four willow seedlings from Lake Mills 6/8/2013 yielded EM colonized root tips. Genetic analysis and sequencing identified the genus *Geopora* as well as two unknown genera from the Pezizaceae.

5.2 Effectiveness of Mycorrhizal Inoculum in Greenhouse Bioassay

5.2.1 Growing Medium: Elwha Silt

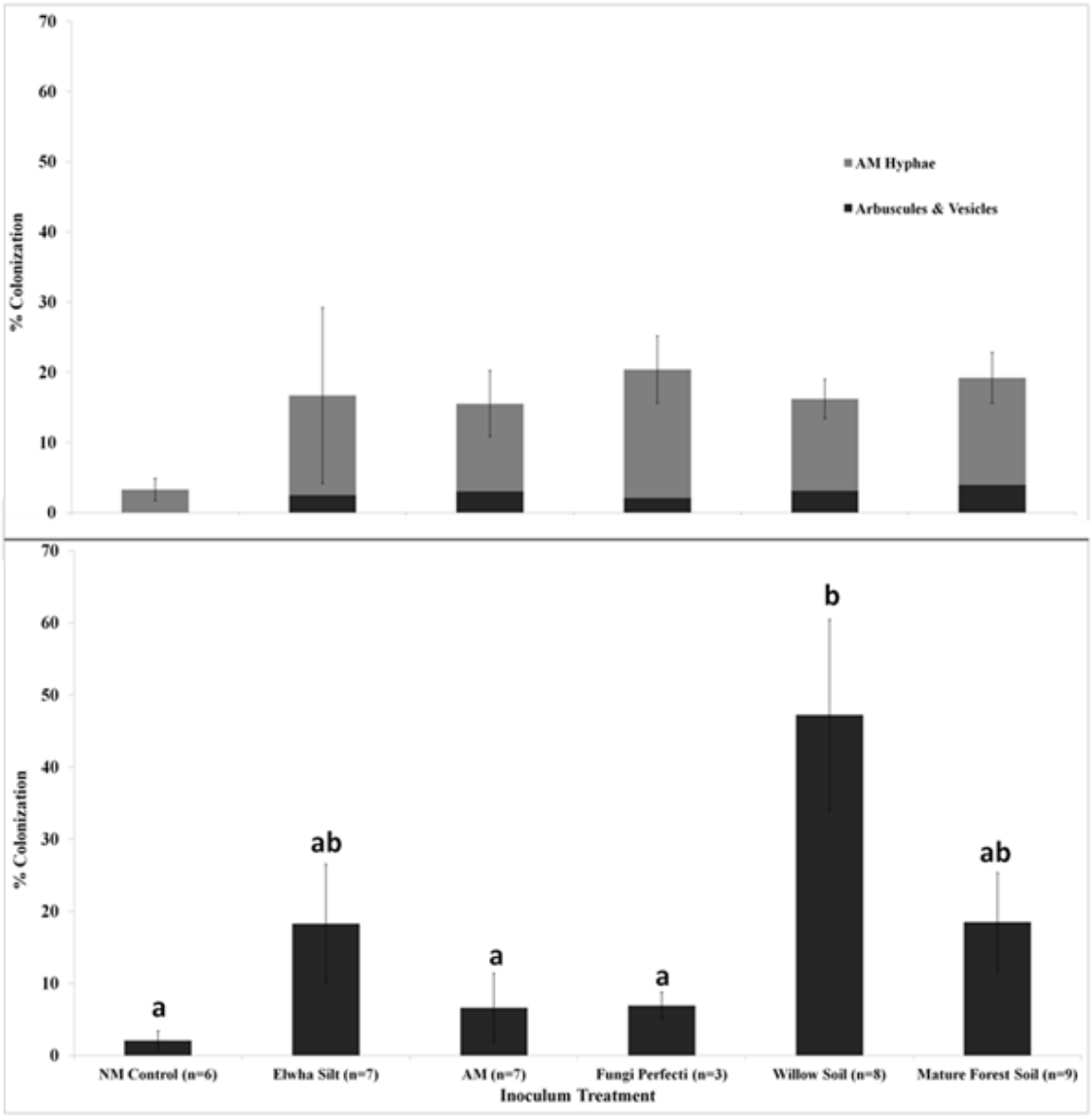

Figure 5: Arbuscular mycorrhizal (top) and basidiomycete/ascomycete (bottom) colonization of willows grown in Elwha Silt. NM control=non-mycorrhizal control and AM=arbuscular mycorrhizal inoculum. Means were compared with one-way ANOVA (α=0.05) and pairwise comparisons made with Holm's adjusted pairwise t-tests (α=0.05).

Whole soil inoculum from mature forest and willow stands produced fully developed ectomycorrhizal root tips in Elwha silt, with willow soil inoculum producing higher colonization than the mature forest (40% and 14%, respectively) ($p<0.05$). No EM root tips were observed from the non-mycorrhizal (NM) control, Fungi Perfecti, AM or Elwha silt treatments (Figure 1.B). Results of genetic analysis failed to sequence DNA from most root tips, but an unknown genus from the Pezizaceae was sequenced from a willow soil inoculated plant. BA hyphae were observed in all treatments with willow soil inoculum producing the highest percent colonization (47%), followed by mature forest (19%) and Elwha silt treatments (18%) (Figure 5). There were low levels of BA hyphae colonization observed in both control and AM treatments which were probably contamination from airborne sources (Stottlemeyer *et al*. 2008). Colonization of AM hyphae ranged from 16% to 20% and 2% to 4% for arbuscules and vesicles but there were no significant differences between any of the treatments ($p>0.05$) (Figure 5). Willows inoculated with mature forest soil had more DSE's than willows inoculated with AM which may have been due to a higher abundance of DSE's in the mature forest soil (Figure 2.B).

5.2.2 Growing Medium: Potting Soil

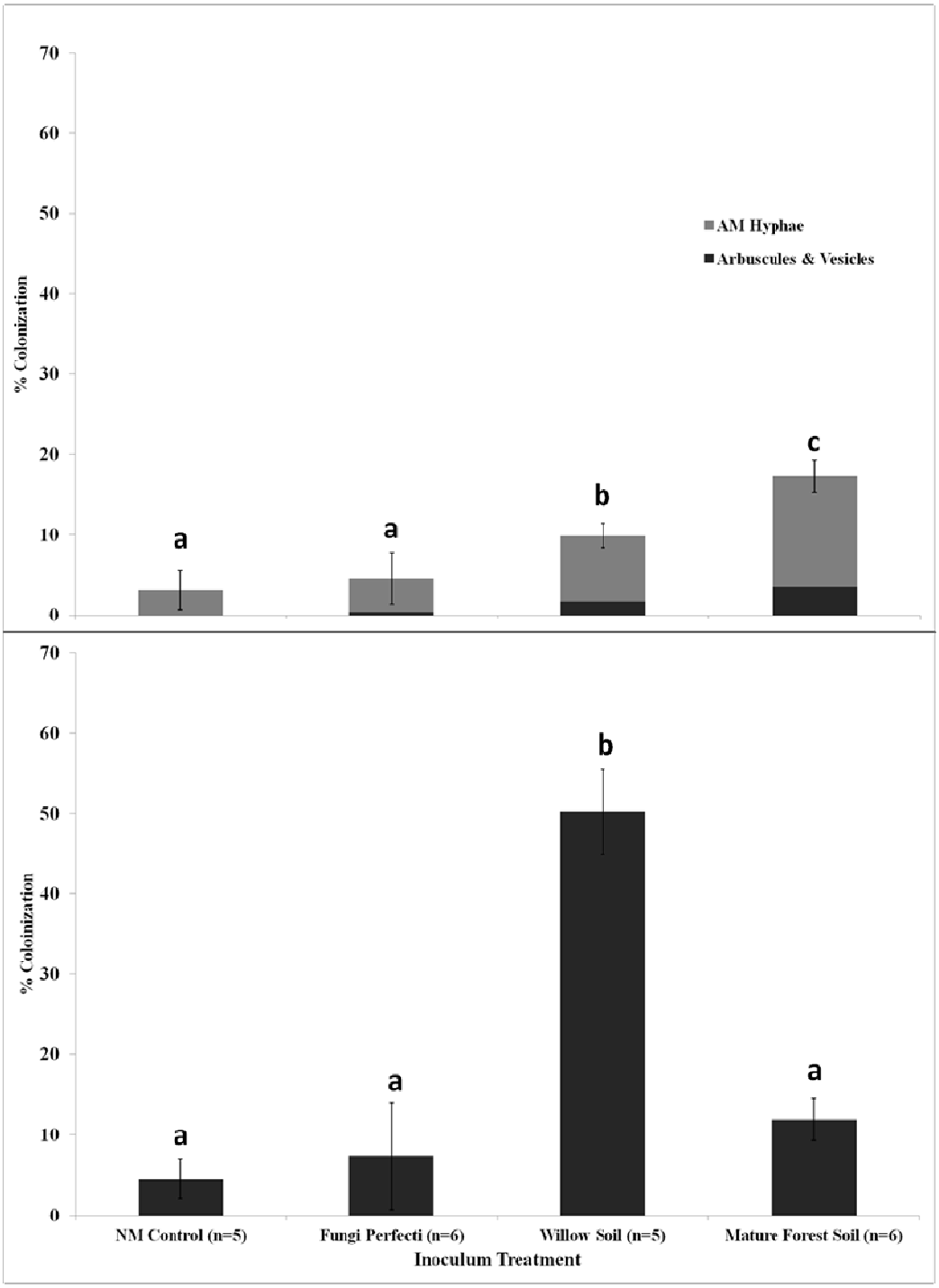

Figure 6: Arbuscular Mycorrhizal (top) and Basidiomycete/Ascomycete (bottom) colonization of willows grown in potting soil. NM control=non-mycorrhizal control and AM=arbuscular mycorrhizal inoculum. Means compared with one-way ANOVA (α=0.05) and pairwise comparisons made with Holm's adjusted pairwise t-tests (α=0.05)

Willow soil inoculation resulted in the highest colonization of EM (42%) but was not significantly different than mature forest soil (22%) and Fungi Perfecti inoculum (10%) ($p>0.05$). No EM root tips were observed on the non-mycorrhizal (NM) control plants. Colonization of EMF in Fungi Perfecti inoculated plants was highly variable between replicates (±9% SE) (Figure 3.B). Results of genetic analysis failed to sequence DNA from most replicates, but *Tuber* sp. and *Sphaeropsis* sp. were sequenced from mature forest inoculated plants. Willow soil inoculated plants had the highest percent colonization of BA hyphae (50%) compared to mature forest (12%) and Fungi Perfecti (7%) treatments ($p<0.05$) (Figure 6). Colonization of AM hyphae ranged from 5% to 17%, arbuscules and vesicles from <1% to 4% (Figure 6) and DSE's from 0% to 1% (Figure 4.B) with no statistically significant differences between treatments ($p>0.05$). Using two-way ANOVA, there was no statistically significant interaction on the formation of mycorrhizae between growing medium and mycorrhizal inoculum type ($p>0.05$) (Tables 11.A-15.A).

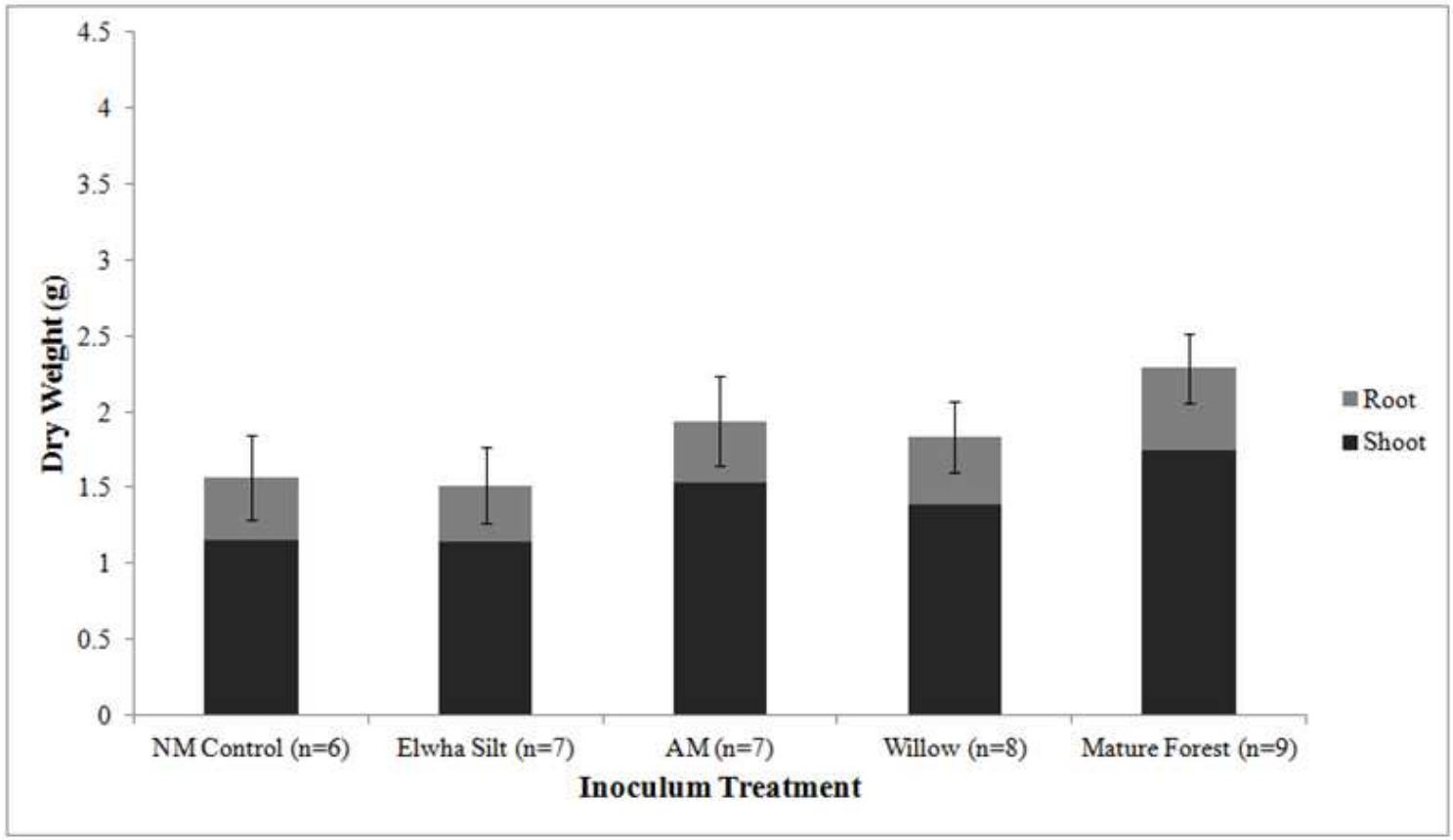

Figure 7: Root and shoot biomass of willows grown in Elwha silt and inoculation with different mycorrhizal treatments. NM control=non-mycorrhizal control. AM=arbuscular mycorrhizal treatment. Groups were compared via one-way ANCOVA (α=0.05).

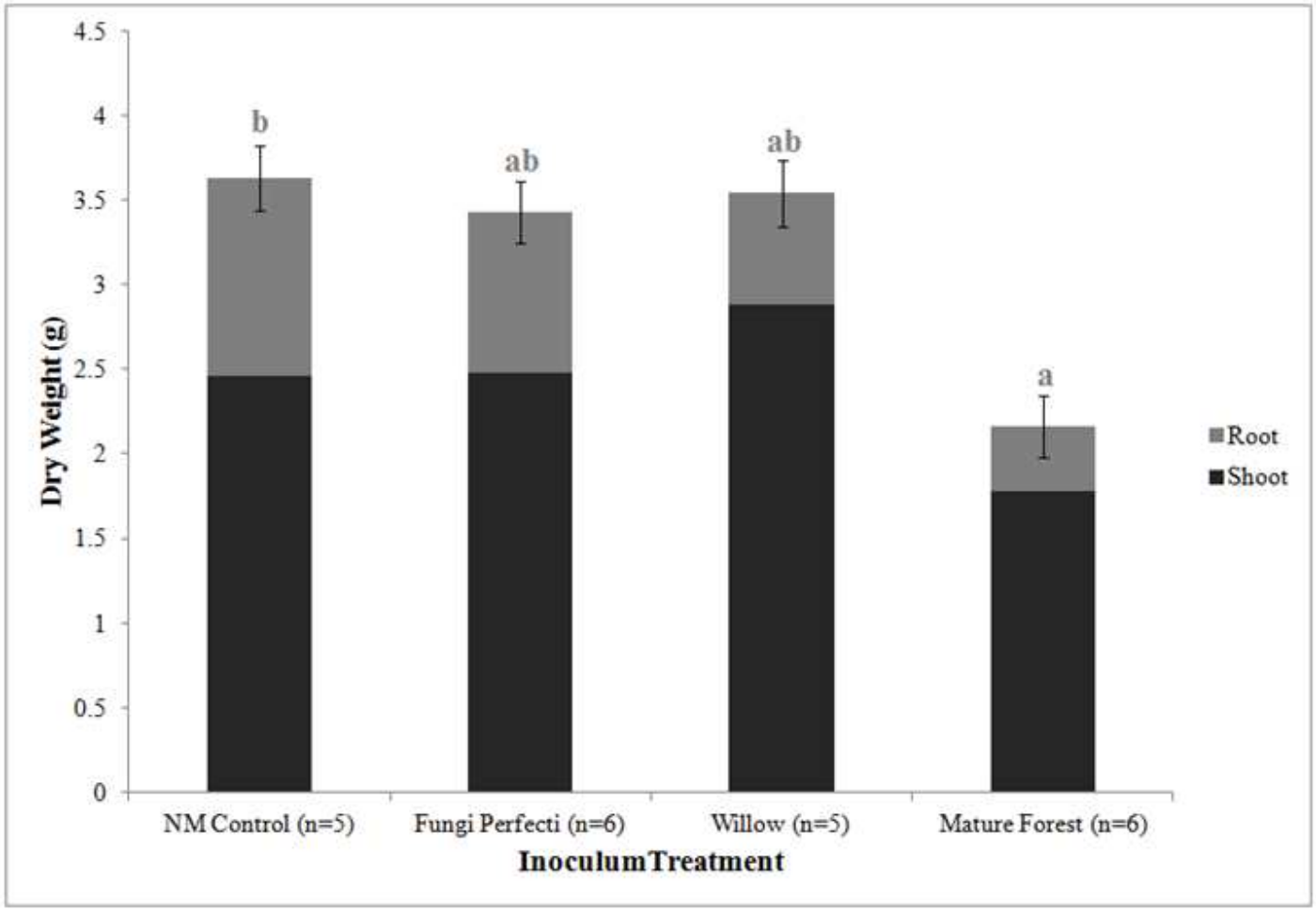

Figure 8: Root and shoot biomass of willows grown in potting soil and inoculation with different mycorrhizal treatments. NM control=non-mycorrhizal control. Groups were compared via one-way ANCOVA (α=0.05). Pairwise comparisons made via Bonferroni adjusted pairwise t-tests (α=0.05).

There were no statistically significant differences in root, shoot, or total biomass between mycorrhizal treatment for willows grown in Elwha silt ($p>0.05$) (Figure 7). Root biomass ranged from 0.38-0.55 g, shoot biomass ranged from 1.16-1.74 g and total biomass ranged from 1.52-2.29 g (Tables 16.A-18.A). Willows grown in potting soil grew about twice as large as willows grown in Elwha silt with the exception of mature forest soil inoculated plants, which grew about the same size as Elwha silt grown plants and had significantly lower root biomass than non-mycorrhizal (NM) control plants (Figure 8). Root biomass ranged from 0.38-1.17 g, shoot biomass ranged from 1.78-2.88 g and total biomass ranged from 2.16-3.63 g (Tables 19.A-21.A).

5.4 Nutrient Analysis of Willow Foliage

There was no treatment effect of mycorrhizal inoculation on total or percent nitrogen and phosphorous for willows grown in Elwha silt ($p>0.05$) (Tables 22.A-23.A & Tables 25.A-26.A). However, all willows showed a large reduction in total nitrogen and phosphorous before and after growth in Elwha silt ($p<0.0001$). Total nitrogen decreased from an average of 2.78 g before growth to 1.16 g after growth (Table 24.A). Total phosphorous decreased from an average of 0.64 g before growth to 0.14 g after growth in Elwha silt (Table 27.A).

6.0 DISCUSSION

6.1 Mycorrhizal Availability of Lake Mills

6.1.1 Spore Density

The observed variability in my spore counts was consistent with pre-dam removal spore count data. The standard error of my spore count data (±11) was similar to that of the pre-dam removal counts (±9) (Chenoweth *et al.* 2011). The high variability suggests that there is a heterogeneous distribution of spores in the lakebed and some patches of sediment

may contain a high density of AM spores while a nearby patch may have few or no spores. Overall, my results suggest that there are more AM spores near the forest and decrease with distance from the forest. However, with high variability in spore density even close to the forest, there may be some patches without adequate inoculum to form mycorrhizae with plants (Figure 9.B). Because of the even patchier distribution of spores further from the forest, the majority of plants will probably not have consistent access to mycorrhizal inoculum and may require inoculation to ensure the formation of mycorrhizae.

6.1.2 Inoculum potential of Elwha Silt

There are viable AM propagules in the Lake Mills basin and the high variability in mycorrhizal colonization was consistent with observed variability in spore counts (Figures 3 & 4) and further supports a heterogeneous distribution of viable mycorrhizal propagules in the soil. There was lower variability in DSE colonization, which suggests that they are more evenly distributed than other fungi. All plants were inoculated with Elwha silt inoculum collected about 20 meters from the forest edge, and likely had a high inoculum density compared to other parts of the Lake Mills basin.

6.1.3 Analysis of Field-Sampled Ectomycorrhizae

Genetic analysis of root tips from four willows collected from the Lake Mills basin in June 2013 yielded 3 ectomycorrhizal fungal taxa: *Geopora sp.* as well as two unknown genera from the Pezizaceae. Although we did not collect enough samples to characterize the EMF community, these results confirm that there are some viable propagules in the Lake Mills basin and that natural willow regeneration are forming EM. However, these willows were all collected within 50 m of the forest edge and it is still unknown if there are any EMF present further out in the lakebed or what their distribution is.

6.1.4 Future Mycorrhizal Availability

At the present time, there may be more AM spores in Lake Mills than I detected in my counts. Spores were extracted and counted from soil collected in March 2012, which was only 6 months after dam removal was initiated and parts of the lakebed became exposed. It is likely that in the time since dam removal, additional spores have entered the lakebed from the surrounding forest. Animals including rodents, deer and insects are important vectors of both AMF and EMF across many different ecosystems and are often critical for the reintroduction of mycorrhizae in primary succession (Maser *et al.* 1978; Allen 1987; Warner *et al.* 1987; Allen *et al.* 1992; Janos & Sahley 1995; Ashkannejhad & Horton 2006). Some animals are utilizing the Lake Mills basin as habitat and therefore are probably vectoring spores. The dispersal of EMF through the consumption of sporocarps and deposition via fecal pellets is relatively well studied (Maser *et al.* 1978; Trappe & Maser 1978; Maser *et al.* 1986; Maser & Maser 1988; Ashkannejhad & Horton 2006). Hypogeous-fruiting EMF, such as truffles, often depend entirely on animals for spore dispersal because they fruit underground and cannot utilize air currents for dispersal (Maser & Maser 1978; Trappe & Maser 1978). One advantage belowground fruiting provides is desiccation resistance and less of a dependence on atmospheric moisture for spore production (Thiers 1984). In my greenhouse bioassay, a species of *Tuber*, a hypogeous fungus, was detected on willows inoculated with mature forest soil. Because the expected primary stressor of the Lake Mills basin is from drought (Chenoweth *et al.* 2011) and much of the lakebed is distant from the forest, these fungi may proliferate because of their desiccation resistant sporocarps and utilization of animals for widespread spore dispersal.

6.2 Colonization Rates of Mycorrhizal Treatments in Greenhouse Bioassay

In Elwha silt, willow soil was the most effective EM inoculum as demonstrated from the highest percent colonization. Willow soil was slightly more effective in the formation of EM compared to mature forest soil. However, mature forest soil would still be the most local source of inoculum for the restoration team, and therefore may be the most practical for implementation. There were no EM root tips detected in Fungi Perfecti plants but some structures consistent with incipient Hartig net and mantle formation were observed on some roots at 40x magnification. Because of high mortality of plants, only 3 replicates were examined for EM root tips. Overall, the two whole-soil inoculums were the most effective in forming AM and EM with willows in Elwha silt. Willows grown in potting soil exhibited similar mycorrhizal colonization rates to those grown in Elwha silt. This means that the Elwha silt does not have a significant effect on the formation of mycorrhizae with willows. The only notable difference in mycorrhizal colonization between the soils was that the Fungi Perfecti inoculum formed EM in potting soil but not in Elwha silt. However, colonization of EM by the Fungi Perfecti inoculum was highly variable; few plants had relatively high colonization while most others had very low or no EM colonization. Additionally, only 3 Fungi Perfecti replicates survived in Elwha silt and thus EM may have been detected if more were examined. One potential cause of the variability in EM colonization could have been the batch of inoculum (Corkidi *et al.* 2004). I used two different bags of inoculum that may have been produced at different times and may have had differences in viability.

6.3 Willow Biomass

6.3.1 Growing Medium: Elwha Silt

There were no significant differences in root or shoot biomass between any treatments. The timeframe for my study was comparable to another greenhouse study that

detected effects of AM and EM in willow (van der Heijden 2001). The lack of differences in biomass between inoculated and uninoculated plants in my experiment indicates that willows were able to establish and grow in sediment from Lake Mills without mycorrhizal inoculation. This implies that willows may be critical for formation of mycorrhizal networks by establishing in sites devoid of mycorrhizae, becoming colonized through stochastic spore dispersal events and then form mycorrhizal networks that may allow other plants to establish (Newman 1988; van der Heijden & Horton 2009). Willows have been shown to do this in primary succession, where they facultatively associated with EMF from wind dispersed propagules, formed mycorrhizal networks, and then subsequently facilitated the colonization of mycorrhizal-dependent plants (Nara & Hogetsu 2004; Nara 2006).

Some plants with specific mycorrhizal requirements are not thriving in Lake Mills. Douglas-fir (*Pseudotsuga menziesii*), an obligate ectomycorrhizal host plant is one of the only restoration plants that have experienced high mortality (Josh Chenoweth-personal communication). Although unconfirmed, a potential cause of mortality may be a lack of access to suitable EMF in Lake Mills. A lack of suitable EMF inhibiting the establishment of EM-dependent conifers has been documented before. In South America, invasions of *Pinus* from plantations have been inhibited due to a lack of suitable EMF critical for establishment and growth (Nuñez *et al.* 2009). Presently, a research team at Peninsula College in Port Angeles, WA is currently examining the effect of inoculation of Douglas-fir on EM colonization and performance after planting in Lake Mills. If the team finds that inoculation improves the survivorship and growth of Douglas-fir, then the revegetation team will need to implement the introduction of inoculum on a large scale to ensure successful establishment and growth of Douglas-fir in the Lake Mills basin.

6.3.2 Growing Medium: Potting Soil

Potting soil grown willows grew about twice as large as Elwha silt grown willows with the exception of the mature forest treatment, which grew smaller than the other treatments and had similar root, shoot and total biomass as willows grown in Elwha silt. The mycorrhizal fungi present in mature forest soil may have interacted with willows differently in potting soil than in Elwha silt. The mycorrhizal symbiosis is not always a mutualism, but is context-dependent, can be concurrently influenced by soil, plant host and mycorrhizal fungi present and can exhibit positive, neutral or negative effects on the host plant (Johnson *et al.* 1997; Jonsson *et al*. 2001; Jones & Smith 2004; Hoeksema *et al.* 2010; Jayne and Quigley 2013). Elwha silt was more stressful for the plants compared to the potting soil as demonstrated by the differences in biomass between the soils. Therefore the contrasting physical and chemical composition of the two soils may have selected less beneficial mycorrhizal fungi from the mature forest soil inoculum and somehow negatively affected the willows (Johnson 1993; Johnson *et al.* 2008).

6.4 Nitrogen and Phosphorous Content of Willow Foliage

There were no differences between treatments of percent and total nitrogen as well as percent and total phosphorous of willows grown in Elwha silt. Willow shoots harvested before planting into Elwha silt had significantly higher percent nitrogen and phosphorous than willows harvested after growth in Elwha silt. Because no potting soil grown willows were analyzed it is unknown whether the Elwha silt or drought stress resulted in the net loss of nutrients in willows. However, it is established that the lack of N and P in the sediment may potentially impede revegetation of the lakebeds due to nutrient stress (Chenoweth *et al.* 2011; Cavaliere & Homann 2012). It should be noted that red alder, a nitrogen fixing species (Hardin *et al.* 2000), is naturally colonizing some areas of Lake Mills. Establishment of alder

could increase the amount of nitrate and other plant available forms of nitrogen for other plants to access (Lavery *et al.* 2004). However, even with nitrogen fixing plants present, the lakebeds will still be phosphorous deficient but the influence on reestablishment of vegetation is unknown at this time.

7.0 CONCLUSION

There are viable EMF and AMF present in the basin of Lake Mills. Analysis of root tips sampled from naturally regenerated willow seedlings in the Lake Mills basin found three distinct EMF genotypes. Willows inoculated with unsterilized silt from Lake Mills in the greenhouse bioassay formed AM. Arbuscular mycorrhizal spore density was highest close to the forest edge and decreased with distance from the forest. There was very high variability in AM spore density which was consistent with preliminary AM spore counts taken before dam removal (Chenoweth *et al.* 2011). Mycorrhizal inoculum potential of the silt was highly variable for the colonization of both AM and basidomycetes/ascomycetes. Colonization of willows by both AMF and EMF from natural inoculum sources was not inhibited by the Elwha silt, and were similar to colonization rates of willows grown in potting soil. Overall, willow soil was the most effective EM inoculum type and would be the best choice for reintroduction of EMF communities for willows. However, I did not detect any positive effect on growth by mycorrhizae in my experiment, and conclude that mycorrhizae may not critical for willow establishment. Consequently, willows will likely be important for primary succession of the lakebeds and may facilitate the establishment of mycorrhizal-dependent plant species through the formation of AM and EM networks from which other plants can establish.

Because of the patchy distribution of mycorrhizal fungi in the basin of Lake Mills, I would suggest to the revegetation team at Olympic National Park that mycorrhizal-dependent restoration seedlings, particularly those in the Pinaceae (i.e. *Pseudotsuga* sp., *Pinus* spp., *Picea* sp., and *Abies* spp.), be inoculated prior to outplanting. Inoculation would ensure that these plants would have the adequate EM symbionts to grow and thus facilitate rapid reestablishment of native forest in the lakebed. I also suggest that outplanted willows be inoculated with mycorrhizal fungi to promote the formation of mycorrhizal networks from which naturally regenerated mycorrhizal-dependent plant species can access. Assessment of the distribution and diversity of EMF, importance of mycorrhizal networks for facilitation of mycorrhizal dependent plants and the effectiveness of mycorrhizal inoculation on performance of willows planted in the basin of Lake Mills would be the subject of further study to expand upon my research.

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APPENDIX

Appendix A. Supplemental Tables

Table 1.A

Relative Abundance of Ectomycorrhizal Root Tips from Willows Grown in Elwha Silt

p<0.0001 $F_{5,34}=23.450$

Treatment	Relative Abundance (%) ± SE	
NM Control	0.00 ± *0.00*	a
Elwha Silt	0.00 ± *0.00*	a
AM	0.00 ± *0.00*	a
Fungi Perfecti	0.00 ± *0.00*	a
Willow Soil	39.77 ± *6.39*	c
Mature Forest Soil	13.71 ± *2.53*	b

Holmes adj. pairwise t-test, $\alpha=0.05$

Table 2.A

Relative Abundance of BA Hyphae from Willows Grown in Elwha Silt

p=0.0008 $F_{5,20}=6.785$

Treatment	Relative Abundance (%) ± SE	
NM Control	2.08 ± *1.33*	a
Elwha Silt	18.29 ± *8.22*	ab
AM	6.60 ± *4.80*	a
Fungi Perfecti	6.94 ± *1.84*	a
Willow Soil	47.22 ± *13.25*	b
Mature Forest Soil	18.52 ± *6.82*	ab

Holmes adj. pairwise t- test, $\alpha=0.05$

Table 3.A

Relative Abundance of Arbuscular Mycorrhizal Hyphae from Willows Grown in Elwha Silt

p=0.164 $F_{5,20}=1.774$

Treatment	Relative Abundance (%) ± SE
NM Control	3.30 ± *1.56*
Elwha Silt	20.37 ± *12.53*
AM	16.67 ± *4.70*
Fungi Perfecti	15.51 ± *4.78*
Willow Soil	16.20 ± *2.80*
Mature Forest Soil	19.21 ± *3.62*

Holmes adj. pairwise t-test, $\alpha=0.05$

Table 4.A

Relative Abundance of Arbuscules and Vesicles from Willows Grown in Elwha Silt

p=0.0984 $F_{5,20}=2.171$

Treatment	Relative Abundance (%) ± SE
NM Control	0.00 ± *0.00*
Elwha Silt	2.10 ± *0.49*
AM	2.47 ± *1.53*
Fungi Perfecti	3.03 ± *0.91*
Willow Soil	3.09 ± *0.47*
Mature Forest Soil	3.94 ± *1.21*

Table 5.A

Relative Abundance of Dark Septate Endophytes from Willows Grown in Elwha Silt

p=0.0023 $F_{5,20}=4.763$

Treatment	Relative Abundance (%) ± SE	
NM Control	1.39 ± *0.75*	ab
Elwha Silt	2.08 ± *1.45*	ab
AM	0.52 ± *0.52*	a
Fungi Perfecti	0.00 ± *0.00*	a
Willow Soil	3.70 ± *1.67*	ab
Mature Forest Soil	6.48 ± *0.24*	b

Holmes adj. pairwise t-test, $\alpha=0.05$

Table 6.A

Relative Abundance of Ectomycorrhizal Root Tips from Willows Grown in Potting Soil

p=0.008 $F_{3,18}=5.369$

Treatment	Relative Abundance (%) ± SE	
NM Control	0.00 ± *0.00*	a
Fungi Perfecti	10.11 ± *9.07*	ab
Willow Soil	41.94 ± *7.60*	b
Mature Forest Soil	21.65 ± *8.05*	ab

Holmes adj. pairwise t-test, $\alpha=0.05$

Table 7.A

Relative Abundance of BA Hyphae from Willows Grown in Potting Soil

p=0.006 $F_{3,4}=21.56$

Treatment	Relative Abundance (%) ± SE	
NM Control	4.51 ± *2.43*	a
Fungi Perfecti	7.34 ± *6.64*	a
Willow Soil	50.28 ± *5.28*	b
Mature Forest Soil	11.93 ± *2.65*	a

Holmes adj. pairwise t- test, $\alpha=0.05$

Table 8.A

Relative Abundance of Arbuscular Mycorrhizal Hyphae from Willows Grown in Potting Soil

p=0.027 $F_{3,4}=9.455$

Treatment	Relative Abundance (%) ± SE	
NM Control	3.13 ± *2.43*	a
Fungi Perfecti	4.55 ± *3.15*	a
Willow	9.88 ± *1.55*	a
Mature Forest	17.28 ± *2.00*	a

Holmes adj. pairwise t-test, $\alpha=0.05$

Table 9.A

Relative Abundance of Arbuscules and Vesicles from Willows Grown in Potting Soil

p=0.0022 $F_{3,4}=37.670$

Treatment	Relative Abundance (%) ± SE
NM Control	0.00 ± *0.00* a
Fungi Perfecti	0.35 ± *0.05* a
Willow Soil	1.77 ± *0.38* b
Mature Forest Soil	3.52 ± *0.05* c

Holmes adj. pairwise t-test, $\alpha=0.05$

Table 10.A

Relative Abundance of Dark Septate Endophytes from Willows Grown in Potting Soil

p=0.537 $F_{3,4}=0.843$

Treatment	Relative Abundance (%) ± SE
NM Control	0.00 ± *0.00*
Fungi Perfecti	0.00 ± *0.00*
Willow Soil	1.39 ± *1.96*
Mature Forest Soil	0.35 ± *0.49*

Table 11.A

Relative Abundance of Ectomycorrhizal Root Tips from Willows Grown in Potting Soil and Elwha Silt

Treatment $p=0.0001$ $F_{3,13}=15.5769$

Soil $p=0.0545$ $F_{1,13}=0.8191$

Interaction $p=0.1073$ $F_{3,13}=2.4777$

Treatment	Relative Abundance (%) ± SE	
NM Control	0.00 ± *0.00*	a
Fungi Perfecti	11.07 ± *11.07*	ab
Willow Soil	50.83 ± *6.09*	c
Mature Forest Soil	14.17 ± *2.82*	b

Holmes adj. pairwise t- test, $\alpha=0.05$

Table 12.A

Relative Abundance of BA Hyphae from Willows Grown in Potting Soil and Elwha Silt

Treatment $p=0.0003$ $F_{3,13}=14.9331$

Soil $p=0.9659$ $F_{1,13}=0.0019$

Interaction $p=0.7788$ $F_{3,13}=0.2557$

Treatment	Relative Abundance (%) ± SE	
NM Control	2.89 ± *1.28*	a
Fungi Perfecti	7.10 ± *2.33*	ab
Willow Soil	48.44 ± *7.48*	c
Mature Forest Soil	15.88 ± *4.15*	b

Holmes adj. pairwise t- test, $\alpha=0.05$

Table 13.A

Relative Abundance of Arbuscular Mycorrhizal Hyphae from Willows Grown in Potting Soil and Elwha Silt

Treatment $p=0.0004$ $F_{3,13}=14.4439$

Soil $p=0.2341$ $F_{1,13}=0.5842$

Interaction $p=0.5089$ $F_{3,13}=0.7186$

Treatment	Relative Abundance (%) ± SE	
NM Control	3.24 ± *1.12*	a
Fungi Perfecti	11.12 ± *3.88*	ab
Willow Soil	13.67 ± *2.25*	ab
Mature Forest Soil	18.44 ± *2.13*	b

Holmes adj. pairwise t- test, $\alpha=0.05$

Table 14.A

Relative Abundance of Arbuscules and Vesicles from Willows Grown in Potting Soil and Elwha Silt

Treatment $p=0.0136$ $F_{3,13}=5.2572$

Soil $p=0.1591$ $F_{1,13}=2.2320$

Interaction $p=0.4783$ $F_{3,13}=0.8769$

Treatment	Relative Abundance (%) ± SE	
NM Control	0.00 ± *0.00*	a
Fungi Perfecti	1.96 ± *1.86*	ab
Willow Soil	2.39 ± *0.52*	ab
Mature Forest Soil	4.08 ± *1.18*	b

Holmes adj. pairwise t- test, $\alpha=0.05$

Table 15.A

Relative Abundance of Dark Septate Endophytes from Willows Grown in Potting Soil and Elwha Silt

Treatment $p=0.047$ $F_{3,13}=3.4762$

Soil $p=0.033$ $F_{1,13}=6.1508$

Interaction $p=0.210$ $F_{3,13}=1.7331$

Treatment	Relative Abundance (%) ± SE	
NM Control	0.93 ± *0.61*	a
Fungi Perfecti	0.00 ± *0.00*	a
Willow Soil	2.78 ± *1.16*	a
Mature Forest Soil	4.03 ± *2.01*	a
Elwha Silt	3.61 ± *1.51*	a
Potting Soil	0.58 ± *0.50*	a

Holmes adj. pairwise t- test, $\alpha=0.05$

Table 16.A

Shoot Mass of Willows Grown in Elwha Silt

Treatment Effect $p=0.081$ $F_{4,36}=2.297$

Corrected Model $p=0.006$ $F_{5,36}=4.029$ Adj $R^2=0.296$

Treatment	Mass (g) ± SE
NM Control	1.159 ± *0.197*
Elwha Silt	1.138 ± *0.179*
AM	1.532 ± *0.207*
Willow Soil	1.387 ± *0.168*
Mature Forest Soil	1.743 ± *0.162*

Table 17.A

Root Mass of Willows Grown in Elwha Silt

Treatment Effect $p=0.699$ $F_{4,36}=0.552$

Corrected Model $p=0.763$ $F_{5,36}=0.515$ Adj. R^2 0.072

Treatment	Mass (g) ± SE
NM Control	0.403 ± *0.103*
Elwha Silt	0.377 ± *0.094*
AM	0.405 ± *0.109*
Willow Soil	0.445 ± *0.088*
Mature Forest Soil	0.547 ± *0.085*

Table 18.A

Combined Root and Shoot Mass of Willows Grown in Elwha Silt

Treatment Effect $p=0.161$ $F_{4,36}=1.766$

Corrected Model $p=0.037$ $F_{5,36}=2.737$ Adj. R^2 0.194

Treatment	Mass (g) ± SE
NM Control	1.562 ± *0.278*
Elwha Silt	1.515 ± *0.253*
AM	1.937 ± *0.293*
Willow Soil	1.832 ± *0.237*
Mature Forest Soil	2.290 ± *0.229*

Table 19.A

Shoot Mass of Willows Grown in Potting Soil

Treatment Effect $p=0.240$ $F_{3,21}=1.544$

Corrected Model $p<0.0001$ $F_{4,21}=9.717$ Adj R^2 =0.624

Treatment	Mass (g) ± SE
NM Control	2.46 ± *0.39*
Fungi Perfecti	2.48 ± *0.36*
Willow Soil	2.88 ± *0.40*
Mature Forest Soil	1.78 ± *0.36*

Table 20.A

Root Mass of Willows Grown in Potting Soil

Treatment Effect $p=0.042$ $F_{3,21}=8.489$

Corrected Model $p<0.001$ $F_{4,21}=8.489$ Adj R^2 =0.588

Treatment	Mass (g) ± SE	
NM Control	1.17 ± *0.19*	b
Fungi Perfecti	0.95 ± *0.18*	ab
Willow Soil	0.66 ± *0.20*	ab
Mature Forest Soil	0.38 ± *0.18*	a

Bonferroni adj. pairwise t- test, $\alpha=0.05$

Table 21.A

Combined Root and Shoot Mass of Willows Grown in Potting Soil

Treatment Effect $p=0.121$ $F_{3,21}=2.236$

Corrected Model $p<0.0001$ $F_{4,21}=12.172$ Adj R^2 =0.680

Treatment	Mass (g) ± SE
NM Control	3.63 ± *0.58*
Fungi Perfecti	3.43 ± 0.54
Willow Soil	3.54 ± *0.60*
Mature Forest Soil	2.16 ± *0.54*

Table 22.A

Percent Nitrogen of Foliage from Willows Grown in Elwha Silt

$p=0.883$ $F_{5,23}=0.3410$

Treatment	% N ± SE
NM Control	1.12 ± *0.06*
Elwha Silt	1.22 ± *0.12*
AM	1.15 ± *0.06*
Fungi Perfecti	1.17 ± *0.10*
Willow Soil	1.06 ± *0.11*
Mature Forest Soil	1.13 ± *0.13*

Table 23.A

Total Nitrogen by Weight of Foliage from Willows Grown in Elwha Silt

p=0.257 $F_{5,23}=1.4120$

Treatment	grams N ± SE
NM Control	1.53 ± *0.15*
Elwha Silt	1.35 ± *0.23*
AM	1.51 ± *0.21*
Fungi Perfecti	1.91 ± *0.13*
Willow Soil	1.44 ± *0.07*
Mature Forest Soil	1.82 ± *0.14*

Table 24.A

Percent Nitrogen Content of Pre- and Post-Treatment Willows Grown in Elwha Silt

p<0.0001 $F_{1,31}=177.9$

Treatment	grams N ± SE	
Pre-Treatment	2.78 ± *0.18*	b
Post-Treatment	1.16 ± *0.04*	a

Table 25.A

Percent Phosphorous of Foliage from Willows Grown in Elwha Silt

p=0.354 $F_{5,23}=1.170$

Treatment	% P± SE
NM Control	0.13 ± *0.01*
Elwha Silt	0.13 ± *0.01*
AM	0.14 ± *0.02*
Fungi Perfecti	0.11 ± *0.02*
Willow Soil	0.17 ± *0.02*
Mature Forest Soil	0.15 ± *0.02*

Table 26.A

Total Phosphorous by Weight of Foliage from Willows Grown in Elwha Silt

p=0.129 $F_{5,23}=1.925$

Treatment	grams P ± SE
NM Control	0.16 ± *0.02*
Elwha Silt	0.15 ± *0.03*
AM	0.18 ± *0.03*
Fungi Perfecti	0.18 ± *0.01*
Willow Soil	0.23 ± *0.02*
Mature Forest Soil	0.25 ± *0.03*

Table 27.A

Percent Phosphorous Content of Pre- and Post-Treatment Willows Grown in Elwha Silt

p<0.0001 $F_{1,31}=322.7$

Treatment	grams N ± SE	
Pre-Treatment	0.64 ± *0.05*	b
Post-Treatment	0.14 ± *0.01*	a

Table 28.A.

List of fungal and bacterial species and amount present in Fungi Perfecti MycoGrow® inoculum

Species	Propagules/g
Glomus intraradices	13
Glomus mosseae	13
Glomus aggregatum	13
Glomus etunicatum	13
Glomus deserticola	2.5
Glomus monosporum	2.5
Glomus clarum	2.5
Glomus brasilianum	2.5
Gigaspora margarita	2.5
Rhizopogon villosullus	208,750
Rhizopogon luteolus	208,750
Rhizopogon amylopogon	208,750
Rhizopogon fulvigleba	208,750
Pisolithus tinctorius	1,252,000
Suillus granulatus	260,930
Suillus punctatapes	260,930
Laccaria bicolor	83,500
Laccaria laccata	83,500
Scleroderma cepa	417,500
Scleroderma citrinum	417,500
Bacillus licheniformis	820,000
Bacillus azotoformans	820,000
Bacillus megaterium	820,000
Bacillus coagulans	820,000
Bacillus pumlis	820,000
Bacillus thuringiensis	820,000
Bacillus stearothermiphilis	820,000
Paenibacillus polymixa	820,000
Paenibacillus gordonae	820,000
Paenibacillus durum	820,000
Azotobacter polymyxa	820,000
Azotobacter chroococcum	820,000
Saccharomyces cervisiae	820,000
Pseudomonas aureofacea	820,000
Pseudomonas florescence	820,000
Deinococcus erythromyxa	820,000
Trichoderma konigii	330,000
Trichoderma harzianum	330,000

Appendix B. Supplemental Figures

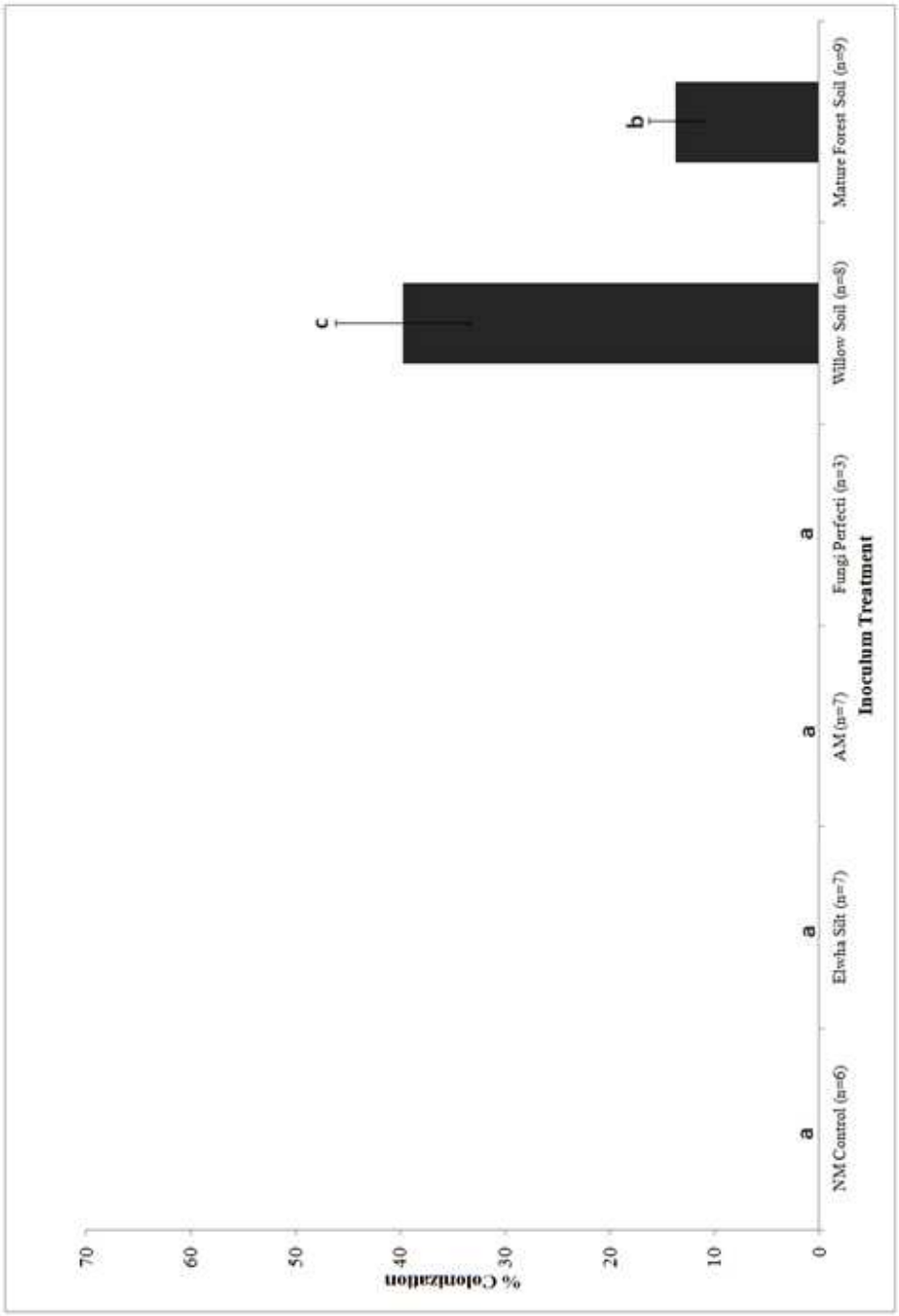

Figure 1.B: Ectomycorrhizal colonization of willows grown in Elwha silt and treated with different sources of mycorrhizal inoculum. NM control=non-mycorrhizal control and AM=arbuscular mycorrhizal treatment. Group means were compared with one-way ANOVA (α=0.05). Pairwise comparisons were done via Holm's adjusted pairwise t-tests (α=0.05)

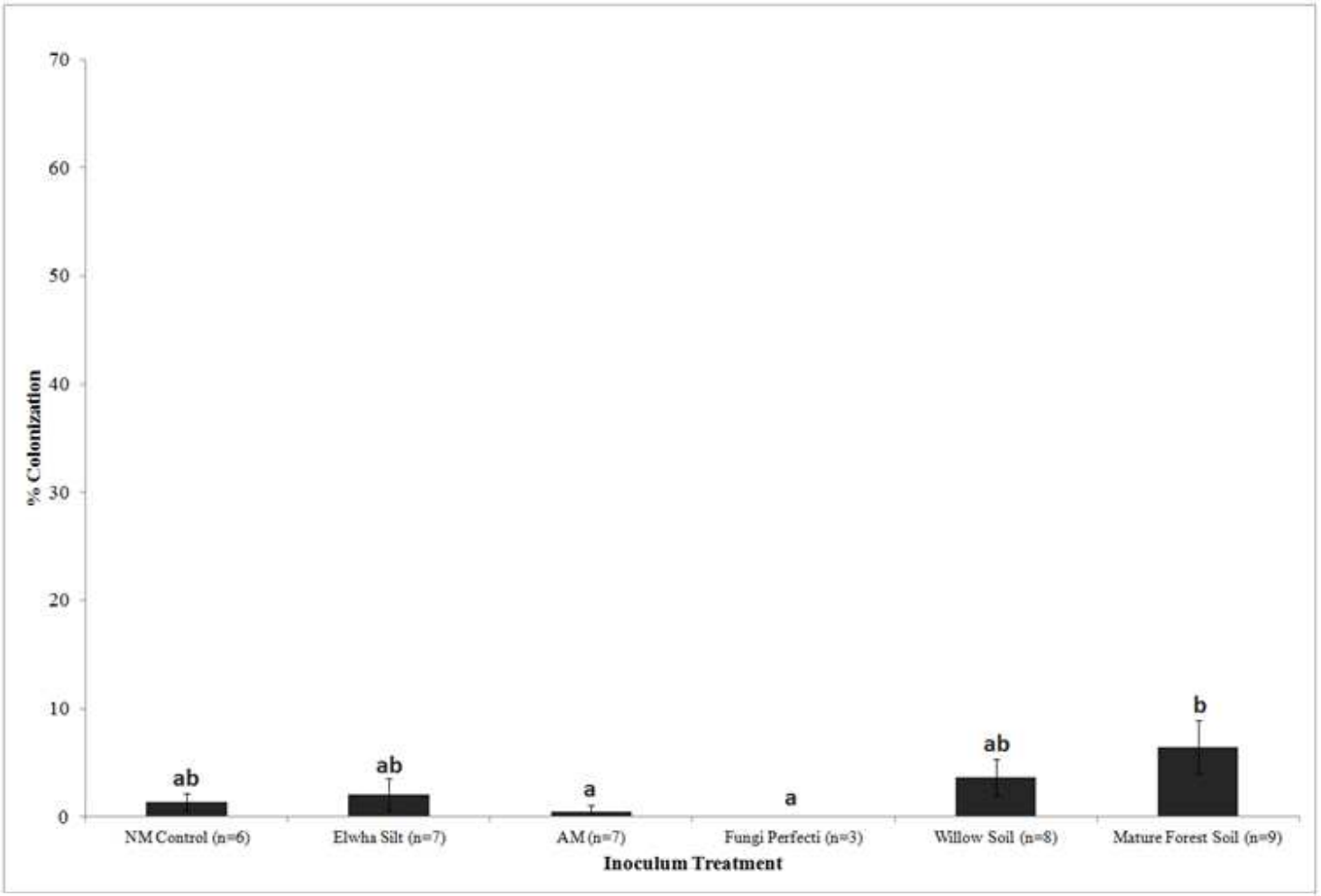

Figure 2.B: Dark septate endophyte (DSE) colonization of willows grown in Elwha silt and treated with different sources of mycorrhizal inoculum. NM control=non-mycorrhizal control and AM=arbuscular mycorrhizal treatment. Group means were compared with one-way ANOVA (α=0.05). Pairwise comparisons were done via Holm's adjusted pairwise t-tests (α=0.05)

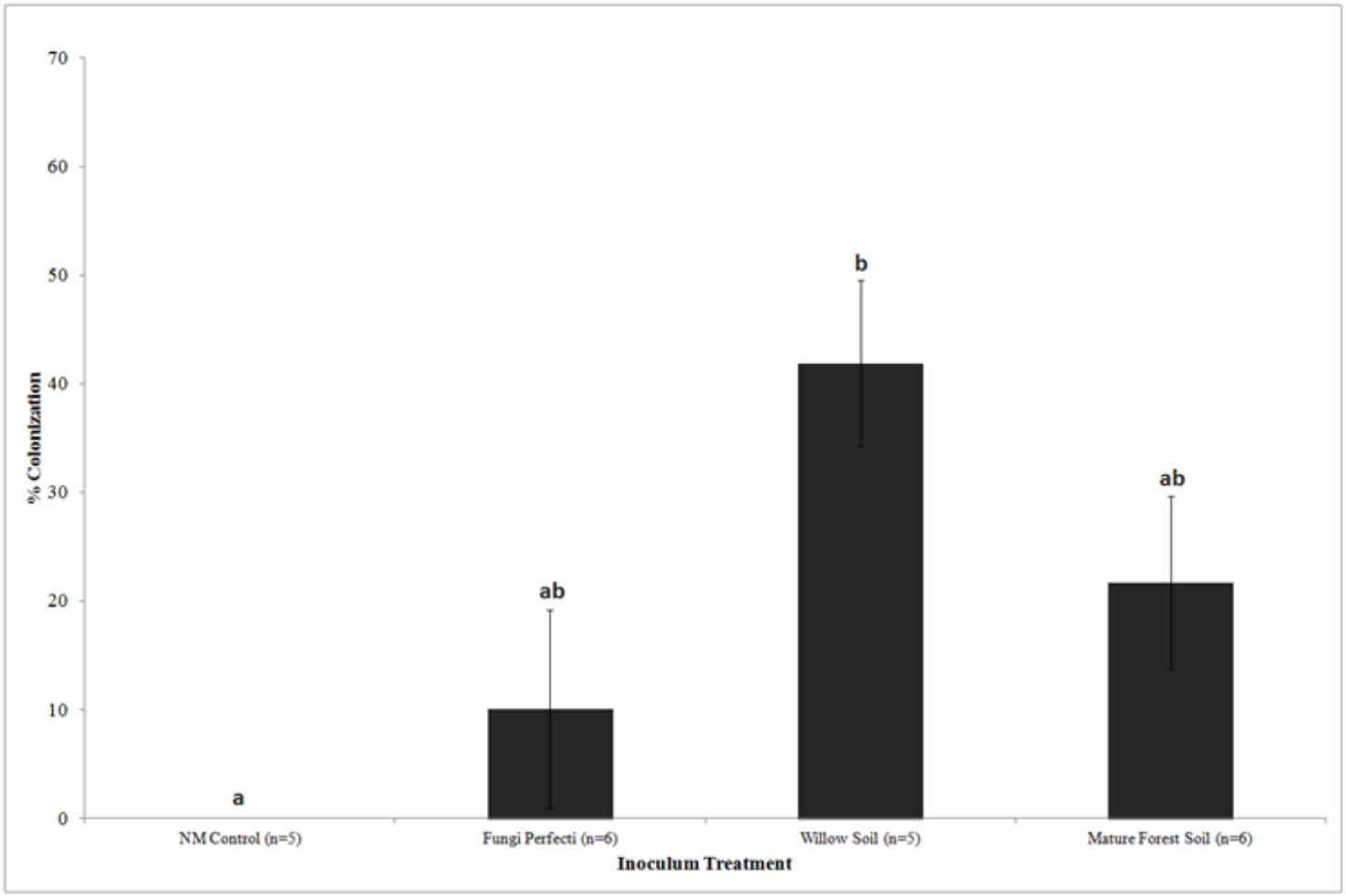

Figure 3.B: Ectomycorrhizal colonization of willows grown in potting soil and inoculated with different types of mycorrhizal inoculum. NM control=non-mycorrhizal control. Group means were compared with one-way ANOVA (α=0.05). Pairwise comparisons were done via Holm's adjusted pairwise t-tests (α=0.05)

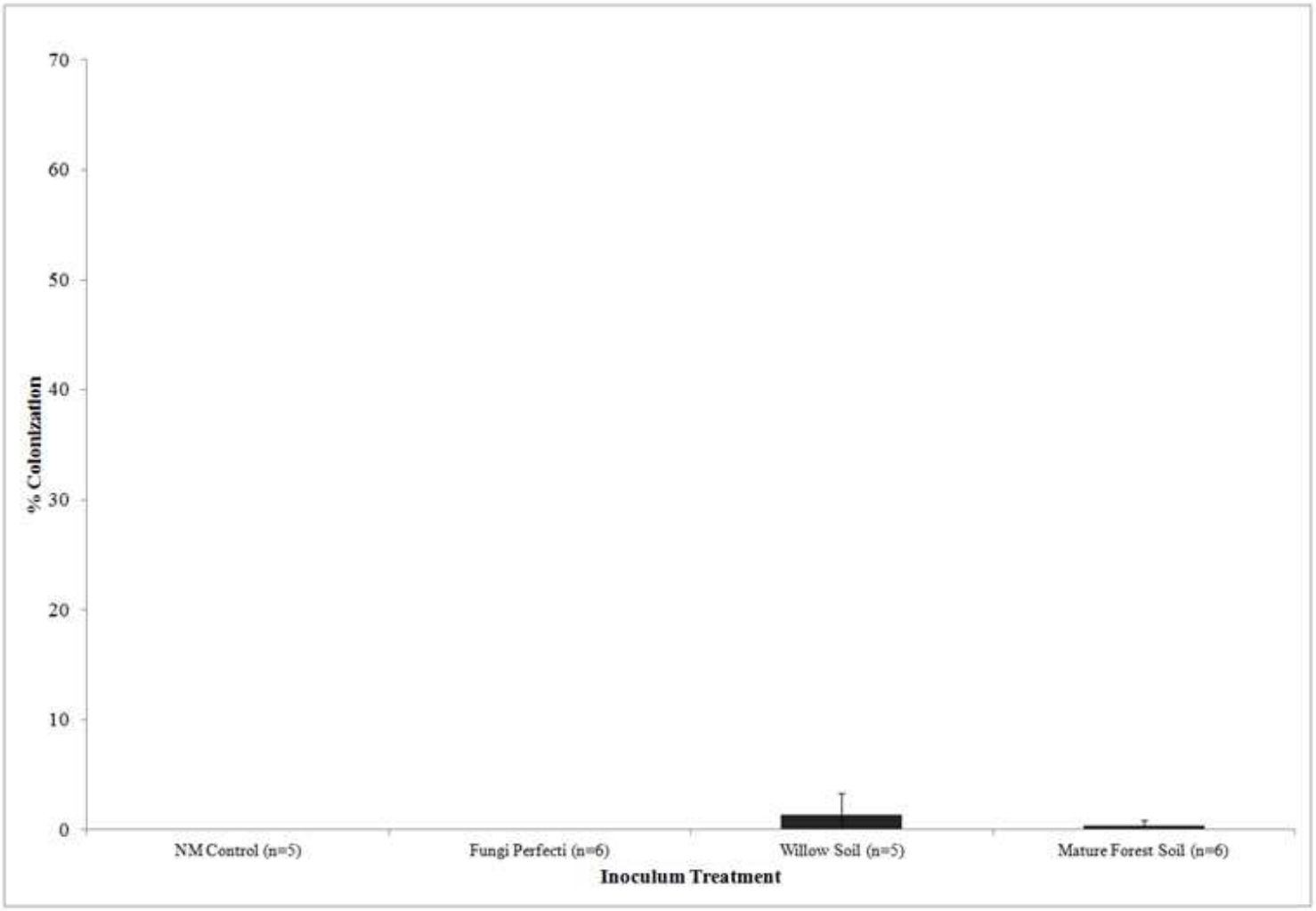

Figure 4.B: Dark septate endophyte (DSE) colonization of willows grown in potting soil and treated with different sources of mycorrhizal inoculum. NM control=non-mycorrhizal control. Group means were compared with one-way ANOVA (α=0.05).

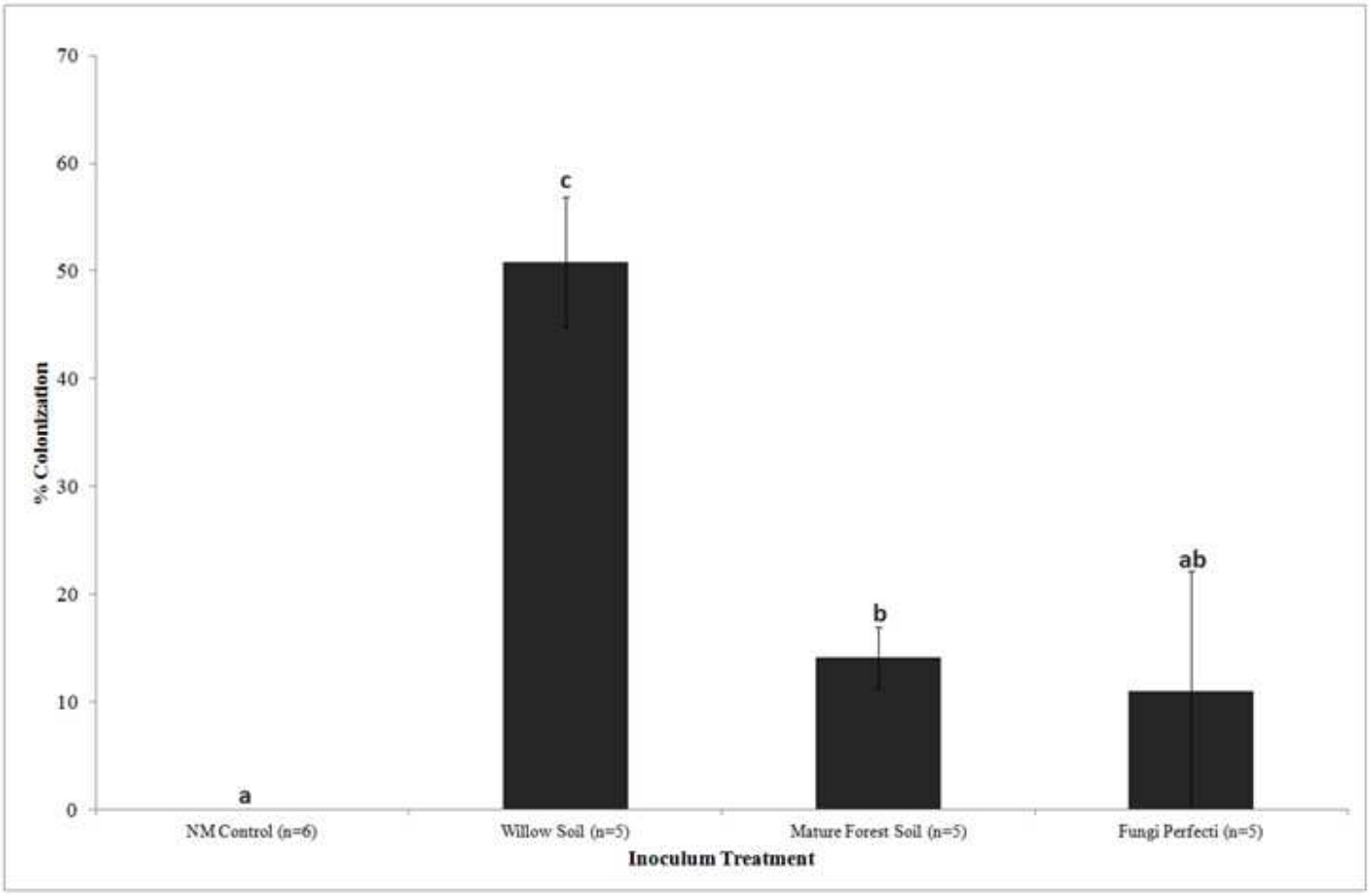

Figure 5.B: Pooled results for ectomycorrhizal colonization of willows grown in Elwha Silt and potting soil and inoculated with different types of mycorrhizal inoculum. NM control=non-mycorrhizal control. Group means were compared with one-way ANOVA (α=0.05). Pairwise comparisons were done via Holm's adjusted pairwise t-tests (α=0.05)

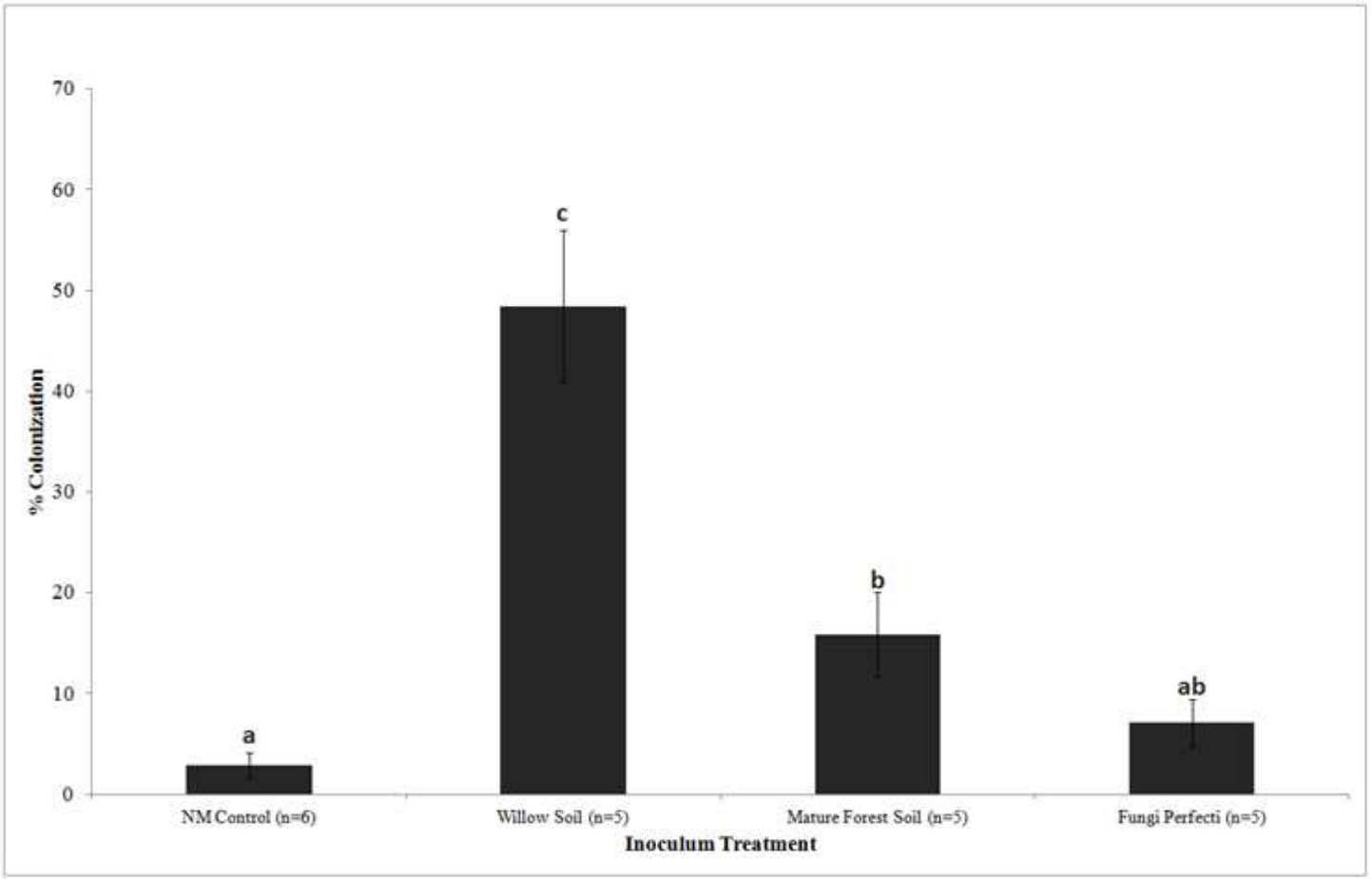

Figure 6.B: Pooled results for basidiomycete and ascomycete hyphal colonization of willows grown in Elwha Silt and potting soil and inoculated with different types of mycorrhizal inoculum. NM control=non-mycorrhizal control. Group means were compared with one-way ANOVA (α=0.05). Pairwise comparisons were done via Holm's adjusted pairwise t-tests (α=0.05)

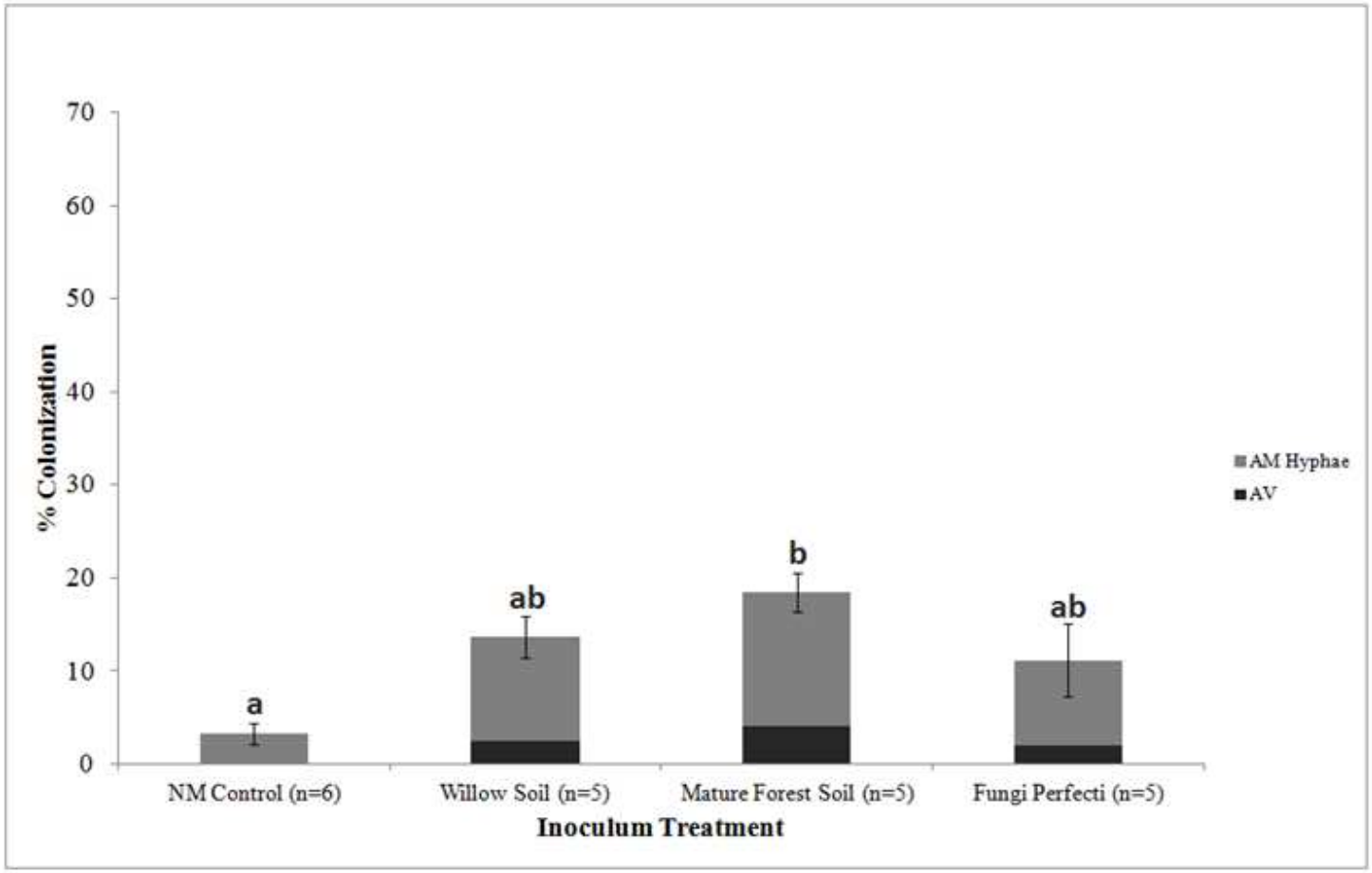

Figure 7.B: Pooled results for Arbuscular mycorrhizal colonization of willows grown in Elwha Silt and potting soil and inoculated with different types of mycorrhizal inoculum. NM control=non-mycorrhizal control. Group means were compared with one-way ANOVA (α=0.05). Pairwise comparisons were done via Holm's adjusted pairwise t-tests (α=0.05)

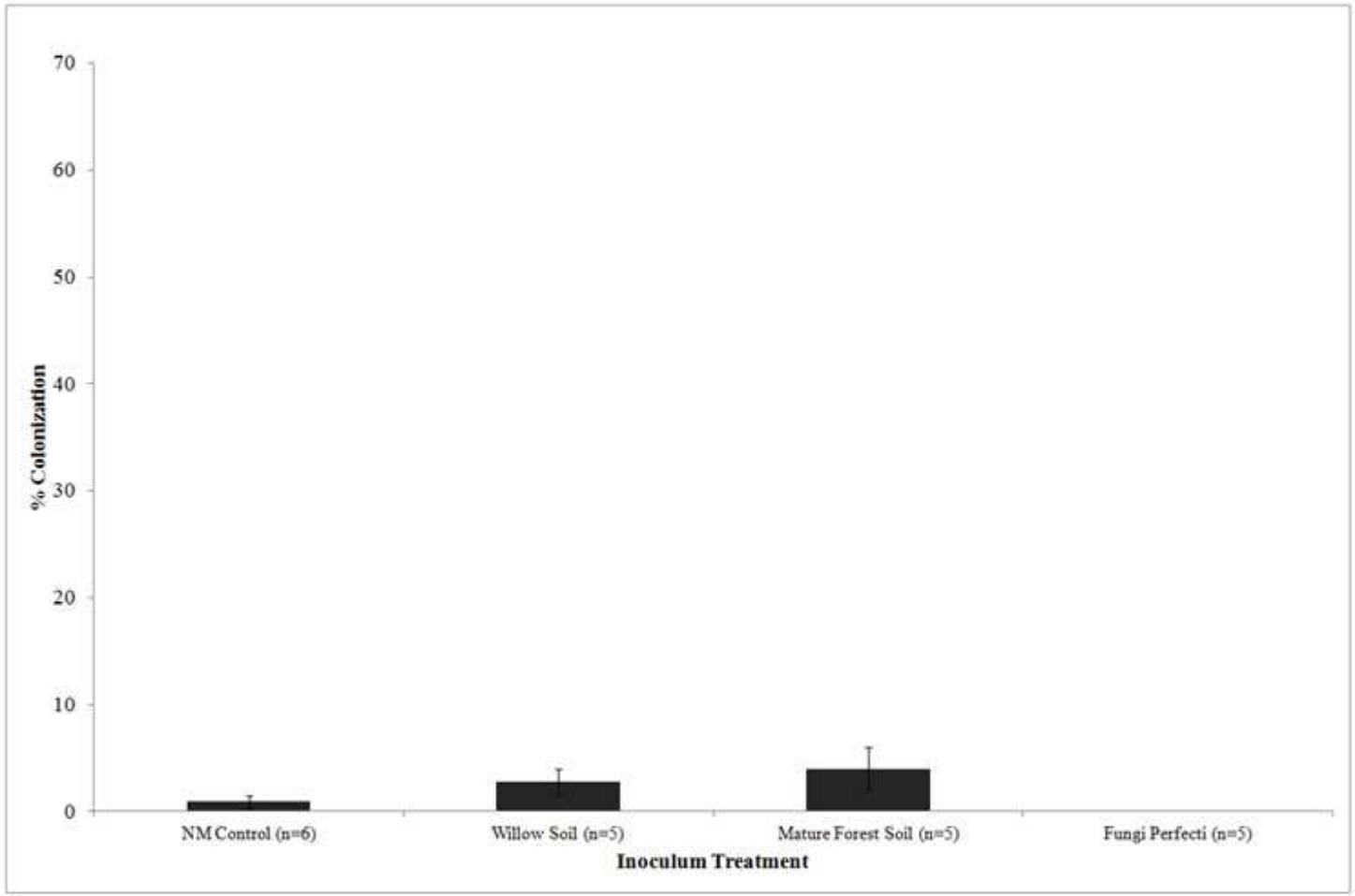

Figure 8.B: Pooled results for dark septate endophyte (DSE) colonization of willows grown in Elwha Silt and potting soil and inoculated with different types of mycorrhizal inoculum. NM control=non-mycorrhizal control.

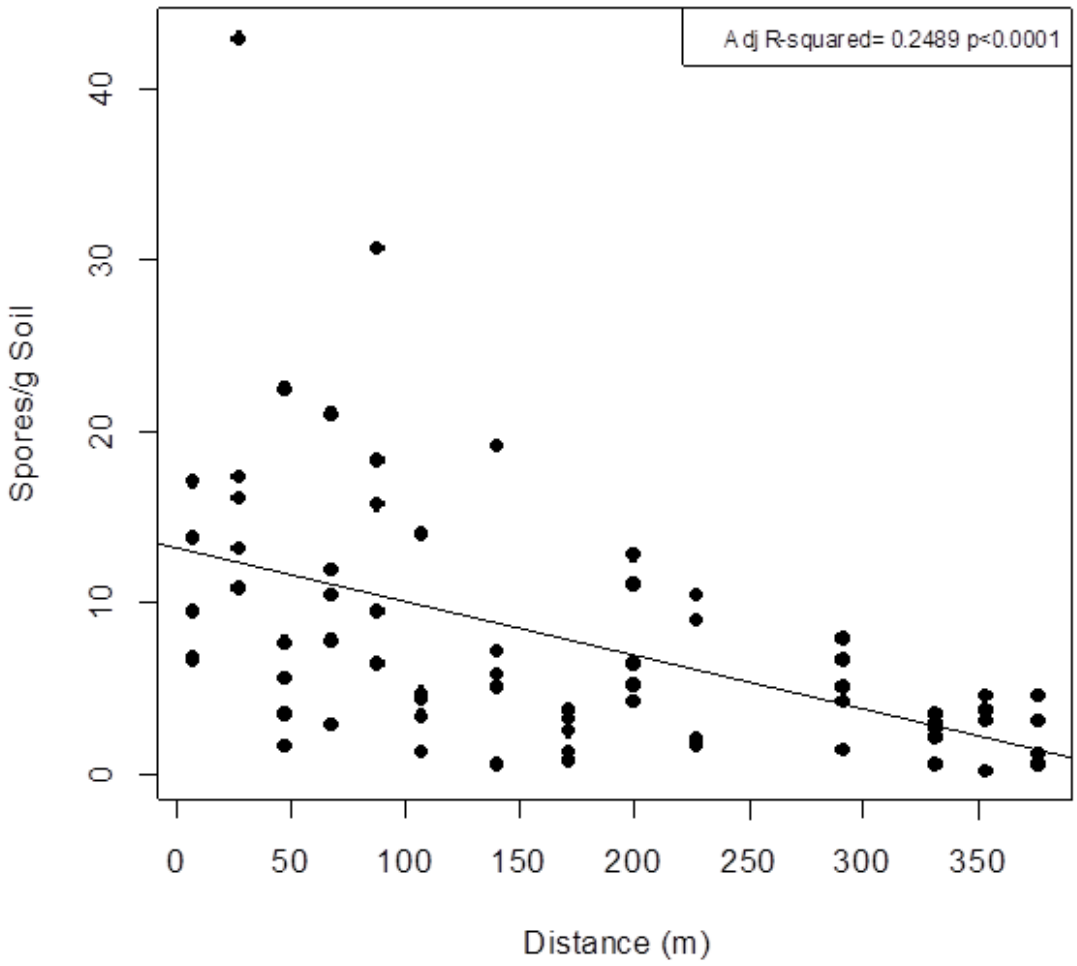

Figure 9.B Arbuscular mycorrhizal spore density present in the basin of Lake Mills along one transect from forest edge to main channel of the Elwha River, March 6 2012 (Figure 1E). Each point represents spore density from a single replicate extraction.

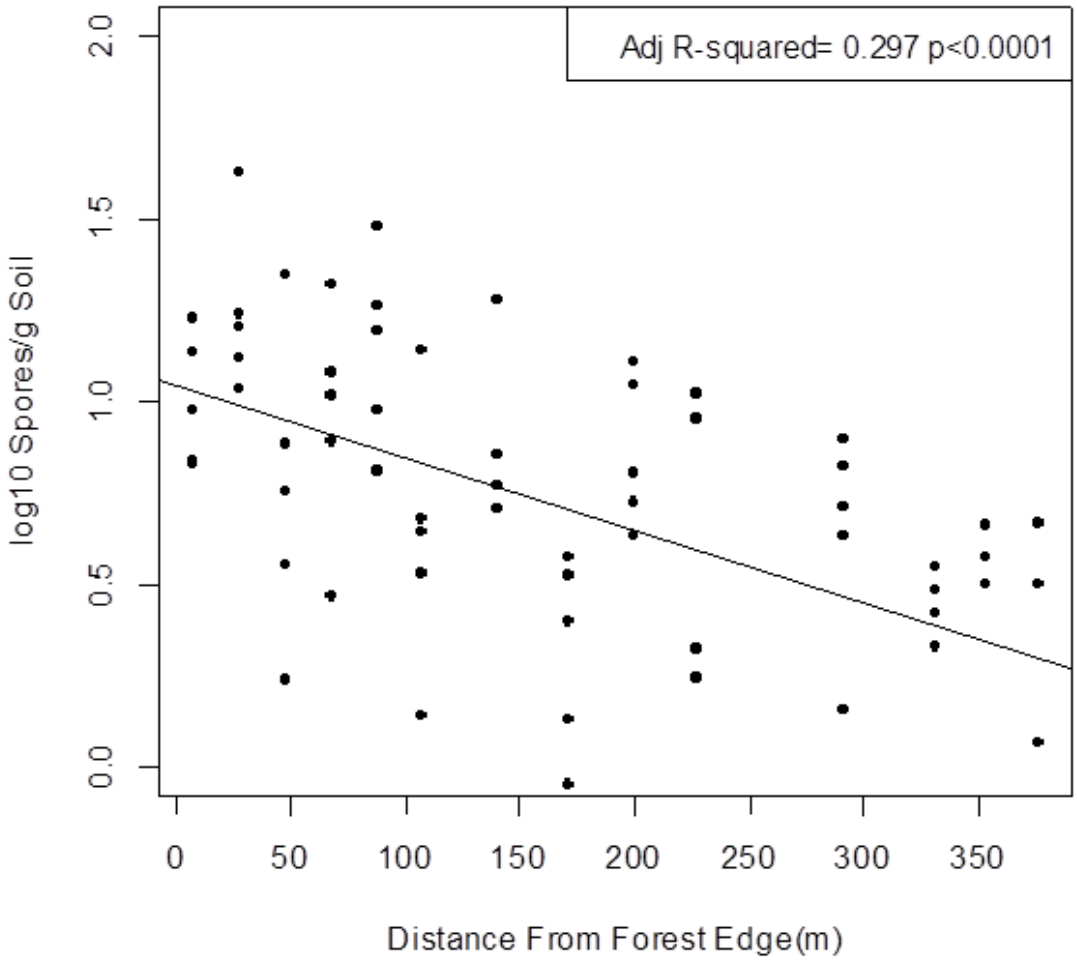

Figure 10.B Log_{10} transformed arbuscular mycorrhizal spore density present in the basin of Lake Mills along one transect from forest edge to main channel of the Elwha River, March 6 2012 (Figure 1E). Each point represents spore density from a single replicate extraction.